Eucalyptus camaldulensis (river red gum) Biogeochemistry: An Innovative Tool for Mineral Exploration in the Curnamona Province and Adjacent Regions

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CHAPTER 1

INTRODUCTION

"Courage and persistence cause success to spring from situations which, on the surface, appear to be absolutely repellent and hopeless"

(GEOLOGY, NO. 8. THE GEOLOGY OF THE BROKEN HILL DISTRICT, E.C ANDREWS, B.A., GOVERNMENT GEOLOGIST).

1.1 INTRODUCTION

Large parts of the Earth's continents, such as Australia, are covered by sediments (i.e. transported regolith) that can obscure the underlying bedrock from surficial geological investigations. In these areas, traditional surficial geochemical methods for mineral exploration, such as rock, stream sediments and soil sampling, have limited effectiveness (Butt *et al.,* 2005). More penetrative approaches however, such as geophysics and drilling, can be expensive and provide limited data coverage. As a result there is increasing need for new methods to efficiently and effectively explore through transported cover. Plant sampling offers a potential means of meeting this need. They colonise two thirds of the Earth's land surface, where they typically develop root systems that extend into, and in some cases through, transported regolith (Drager & Lauer, 1967). The roots are involved in chemical exchanges with the regolith substrate, and translocate chemical characteristics into surficial plant organs that can be readily accessible in sampling programs. The chemical analysis of plant organs can therefore potentially allow for the efficient surficial biogeochemical expression of buried geology.

Eucalyptus camaldulensis Dehnh. var *camaldulensis* (river red gum) is one of the most widely distributed tree species within Australia (Figure 1.1). They mostly form belts or stands with minimal woody understorey along the fringes and within watercourses throughout much of arid and semi-arid Australia (Beadle, 1981). An important characteristic of *E. camaldulensis* is the development of an extensive and deep root system. The root system of a mature *E. camaldulensis* extends at least 20 m in the horizontal direction (Dexter, 1967) and greater than 10 m vertically (Davies, 1953), although this study has found examples of lateral root extension > 100 m. *E. camaldulensis* can therefore have a biogeochemical sampling area of > 4000 m^3, with potential for element uptake via their roots from: the adjacent stream sediments; the shallow ground water aquifers within the alluvial sediments; and, buried bedrock or saprolite (Figure 1.2).

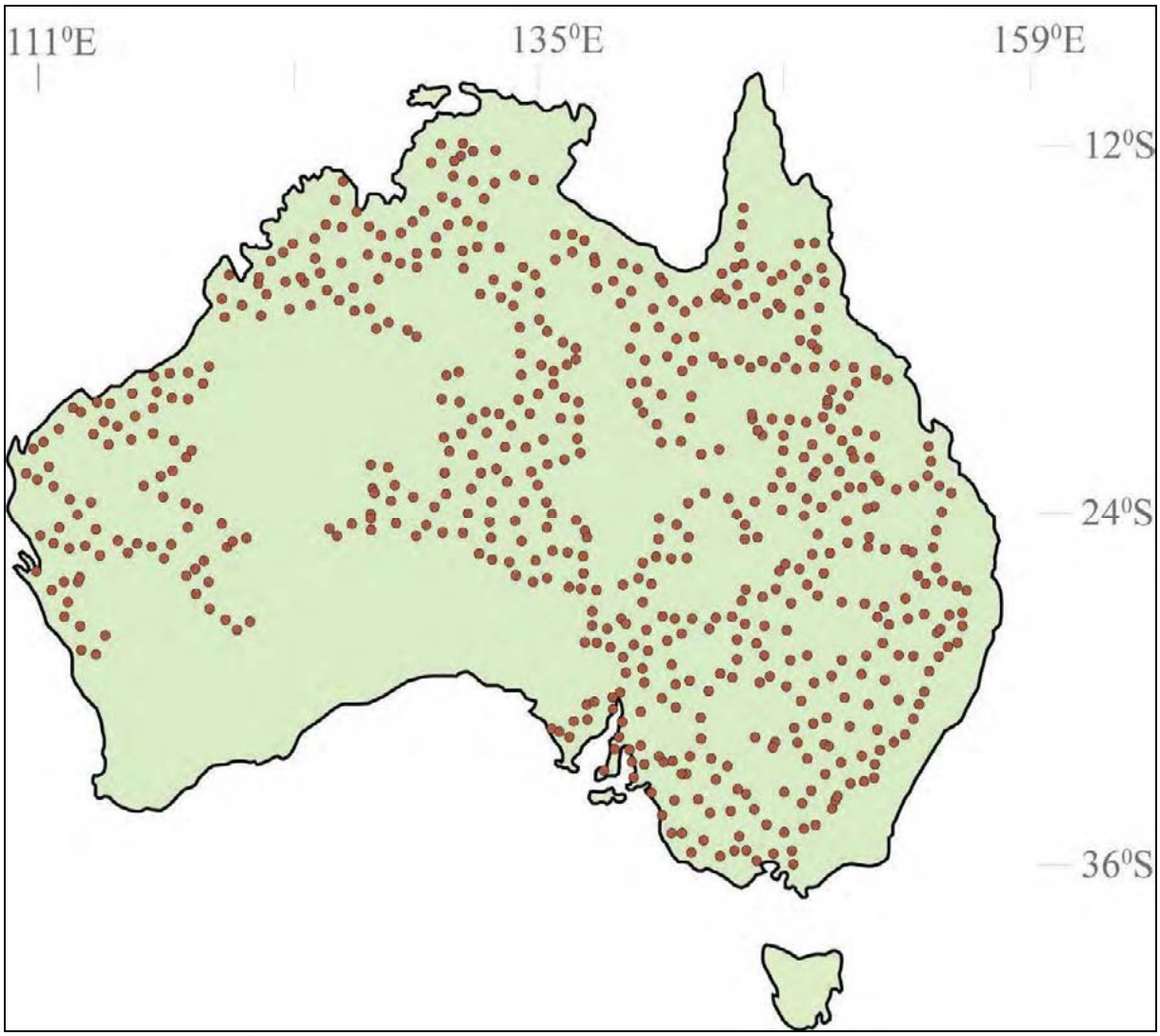


Figure 1.1: The distribution of *E. camaldulensis* within Australia.

Despite its prevalence in the Australian landscape, very little is known about the biogeochemical characteristics and behaviour of *E. camaldulensis*, in particular its trace element and metal characteristics. A small, preliminary biogeochemical survey of *E. camaldulensis* by Dann (2001) provided some encouraging results for mineral exploration programs, yet its wide sample spacing raised more questions than answers about the relationship between these trees and the underlying substrate. This limited understanding has resulted in *E. camaldulensis* not previously being utilised in mineral exploration programs. The widespread and abundant occurrence of *E. camaldulensis* in regolith-dominated terrains (in particular the basin margins of highly prospective regions) however, makes them a strong candidate for development as a biogeochemical sampling medium. This study provides the first detailed biogeochemical study of *E. camaldulensis*, ranging from sub-microscopic to landscape scale studies, with the intention of assessing their potential for development as a mineral exploration sampling medium.

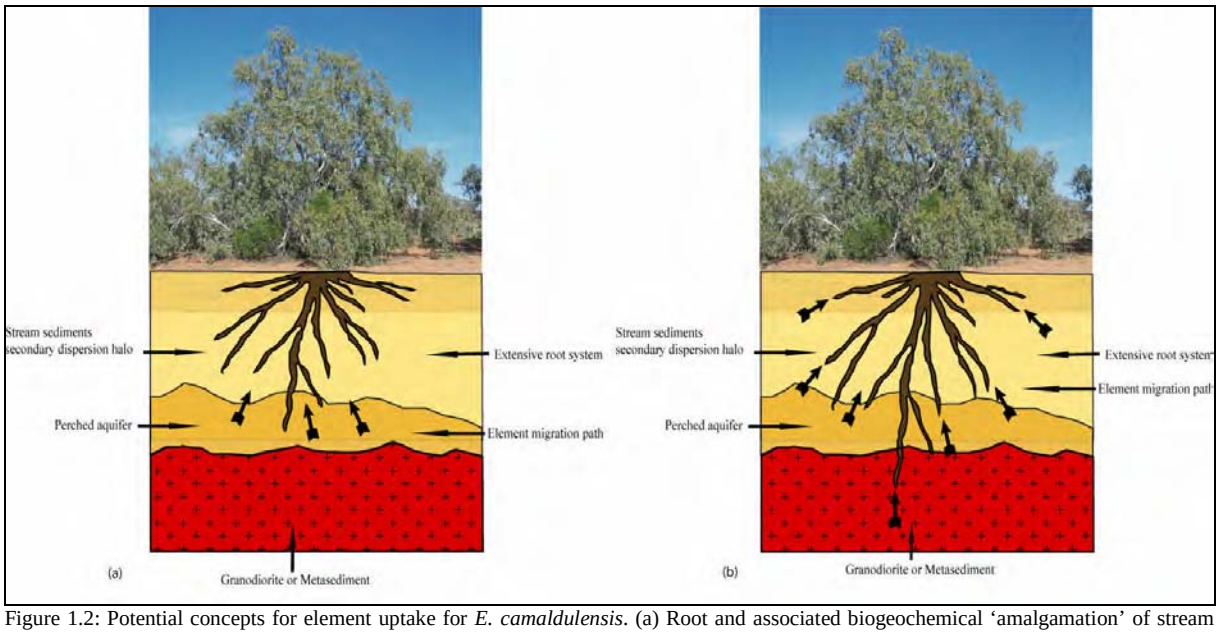


Figure 1.2: Potential concepts for element uptake for *E. camaldulensis*. (a) Root and associated biogeochemical 'amalgamation' of stream sediments and possible hydrogeochemistry of perched aquifers within the transported regolith providing chemical vectors reflecting the underlying bedrock chemistry; and, (b) root penetration to the underlying bedrock

1.2 REVIEW OF THE POTENTIAL REGIONAL (< 100 km^2) APPLICATION OF PLANT BIOGEOCHEMISTRY

The utilisation of plants in regional biogeochemical mineral exploration programs (i.e. 1 sample per 100 km^2 or more) have been successfully employed in many parts of the world. The following provides a brief overview of some of these applications.

1.2.1 International Studies

Malyuga (1964), Boyle (1967), Fortescue & Hornbrook (1969), Brooks (1972), Kovalevskii (1979, 1987) and Dunn (2007) have compiled reviews on the application of biogeochemistry for mineral exploration with an emphasis on northern hemisphere case studies. The application of biogeochemistry for mineral exploration and the associated techniques used in many northern hemisphere countries, such as Canada, are at a relatively mature stage of development. From 1940-1966 the North American 'father of biogeochemistry', Harry Warren, initiated a long progression of studies, which resulted in the rapid progress of biogeochemistry in British Colombia. He continued to augment these studies for another 30 years (long into his retirement), but it was during this earlier period that Warren and his associates undertook much of their fundamental work in recognizing and documenting the relationship between plant chemistry and buried mineralisation. This valuable research raised the credibility of biogeochemistry, or as Professor Warren expressed, it developed "from common scepticism, to a general belief that when employed correctly it can be a valuable tool for mineral exploration" (Brooks, 1972). Table 1.1 shows a list of plant species and organs, location and mineralisation targets that have featured in the application of some regional biogeochemistry sampling programs in the northern hemisphere.

Table 1.1: A list of plant species that have been studied and used in biogeochemical surveys in the Northern hemisphere.

Location	Plant species	Sample collected	Mineral/Element sought	Analytical Method	Reference
Calabria (Southern Italy)	*Pinus laricio & Spartium junceum*	Twigs, herbs	Sphalerite, minor galena, pyrite, chalcopyrite & fluorite	X-ray fluorescence	Crisci *et al.* (1982)
California (Los Angeles)	*Larrea tridentata, Ambrosia chamissonis & Artemisia tridentata*	Leaves & twigs	Gold-hosted in quartz monzonites	INAA	Busche (1989)
Canada (Ontario)	*Betula papyrifera & Populus tremuloides*	Bark, leaf & second tear twigs	Silver	AAS	Hornbrook (1970)
Canada (Northern Saskatchewan)	*Pinus banksiana, Picea mariana, Ledum groenlandicum, Vaccinium spp.*	Twigs, needles, wood, bark cone & roots	Uranium-nickel	NAA AAS	Walker (1979)
Canada (Northern Saskatchewan)	*Pinus banksiana, Picea mariana, Ledum groenlandicum and Chamaedaphne calyculata*	Twigs and needles	Uranium	INAA	Dunn (1981)
Canada (Saskatchewan)	*Populus tremula L. Cornus spp. & Corylus spp.*	Twigs	Kimberlite (Diamonds)	INAA, ICP-ES & ICP-MS	Dunn (1992)
Canada (Nova Scotia)	*Abies balsamea*	Twigs	Gold	INAA & NAA	Rogers & Dunn (1993)
Egypt (Abu Swayel & Haimur)	*Acacia raddiana & Acacia ehrenbergianana*	Twigs, stem & roots	Complex copper-nickel sulphide	Polarographic & calorimetric	El Shazly *et al.* (1970)
Morocco	*Anvillea garcinii subsp. Radiata, Ononis natrix, Lavandula multifidia, Convolvulus trabutianus & Artemisia herba-alba*	Twigs	Copper-Nickel Arsenide	AAS	Dunn *et al.* (1996)
NE Turkey (Trabzon)	*Corylus avellana*	Leaves & twigs	Massive sulphide deposit (Cu, Zn Pb anomalies)	Flame atomic Absorption spectrometry	Akcay *et al.* (1998)
Northern Cordillera	*Pinus ponderosa & Abies concolor*	Twigs, needle & wood	Disseminated gold	Graphite/furnace AAS ICP & NAA	Ashton & Riese (1989)
Papua New Guinea	*Astronidium palauense*	Roots, bark, twigs, & flowers	Gold-copper	INAA	Mc Innes *et al.* (1996)
Sri Lanka	*Glycosmis mauritiana & Pterospermum*	Leaves	Copper-Magnetite	ICP	Brooks *et al.* (1985)
Wadi (Israel)	*Fagonia mollis & Palicaria undulata*	Whole plant	Copper-bearing faults	Multi-element DC carbon arc (OES)	Bogoch & Brenner (1984)

1.2.2 Australian Studies

In Australia, the application of biogeochemistry for mineral exploration has been on a much less extensive scale and with less co-ordination (Brooks, 1972). This is particularly shown by the lack of Australian case studies or results in the recent overview of plant biogeochemical applications to mineral exploration by Colin Dunn (Dunn, 2007). The earliest published account in Australia is of the geobotanical and biogeochemical indicator species, the pink copper plant *Polycarpaea spirostylis*, as recorded by Mr. Herschel Babbage in 1858 (Skertchly, 1897). This plant appeared to grow in close association with base metal mineralisation (Nicholls, 1966) and became recognised as a Cu geobotanical indicator in northwestern Queensland (Cole, 1965, Correll & Taylor, 1974). The first detailed account of vegetation being chemically analysed for use in mineral exploration in Australia took place over 40 years ago by Monica Cole (1965). Over the following 25 years the development of

biogeochemical exploration in Australia was sporadic, with biogeochemical studies taking place as components of larger PhD studies (e.g. Hall, 1971), and some limited and typically propriety studies undertaken by mineral exploration companies (e.g. Pasminco, 1970). An increase in the application of biogeochemical exploration has taken place in the last few decades (e.g. Cruikshank & Pyke, 1986; Cole, 1991; Lintern *et al.*, 1997; Arne *et al.*, 1999), perhaps largely reflecting the shift in focus of mineral exploration towards settings predominately covered by transported regolith as well as due to improvements in chemical analysis techniques. The Cooperative Research Centre for Landscape Environments and Mineral Exploration (CRC LEME) recently investigated the application of biogeochemistry for mineral exploration and a wide range of plants were tested (Hill, 2003; Hill & Hill, 2003; Hulme, 2006; Anand *et al.*, 2007). Table 1.2 shows a list of plant species, organ collected, location and associated mineral commodity associated with some regional biogeochemistry sampling programs in Australia and New Zealand. This shows that a range of different species have been trialled in different regions, however, a concerted effort towards characterising and understanding any single species has been lacking. It also shows that, perhaps with the exception of Monica Cole, there has not been a consistent and sustained testing and publication of results from this approach by a single researcher. A detailed study of the biogeochemistry of a single plant species, such as *E. camaldulensis*, is therefore timely.

Table 1.2: A list of plant species that have been studied and used in biogeochemical surveys in Australia.

Location	**Plant species**	**Sample organ**	**Mineral/Element sought**	**Analytical Method**	**Reference**
New South Wales (Broken Hill)	*Ptilotus obvatus* & *Prostanthera striatiflora*	Leaves & flowers	Lead lodes (little Broken Hill), Apollyan valley (Angus mine & Thackaringa	Not stated	Cole (1965)
South Australia (Pernatty & Ediacara)	*Ptilotus obvatus* & *Prostanthera striatiflora*	Leaves & flowers	Copper and lead	Not stated	Cole (1965)
Queensland (Dugald River area)	*Polycarpaea glabra, Eriachne mucronata, Bulbostylis*	Stems, leaves, flowers & fruit	Lead-Zinc & copper mineralisation	Not stated	Cole (1965)
Northern Territory (Bulman –Weimool Springs area)	*Gomphrema canescens, Polycarpaea spirostylis & Trianthema rhyncocalyptra*	Leaves	Lead-Zinc & copper mineralisation	Not stated	Cole (1965)
Western Australia (Widgiemooltha)	*Hybanthus floribundus (Ni accumulator)*	Unknown	Gold fields (Ni mineralisation)	AAS	Cole (1973)
Western Australia (Mt Thirsty)	*Casuarina campestris & Ricinocarpus stylosus*	Unknown	Gold fields	AAS	Cole (1973)
South Australia (Stuart Shelf)	*Acacia spp. & Callitris columellaris or Callitris glauca*	Twigs & leaves	Copper sulphides	Emission spectrographic, AAS	Rattigan *et al.* (1977)
Northern Territory (Ranger One)	*Eucalyptus miniata*	Leaves & twigs	Uranium	XRF & AAS	Cruikshank & Pyke (1986)
Queensland (Thalanga)	*Eucalyptus shirleyii*	Leaves, second year twigs and outer	Zinc-lead-copper (Charters Tower)	AAS	Cole (1991)

Location	Plant species	Sample organ	Mineral/Element sought	Analytical Method	Reference
		bark			
Queensland	*Eucalyptus affin, Polycarpa & Melaleuca sp & Petalostigma quadriloculare*	Leaves, 2nd year twigs & outer bark	Zinc-lead-copper	AAS & ICP-AES	Cole (1991)
Victoria	*Eucalyptus ssp., Pinus radiata & Cassinia aculeata*	Bark, twigs, leaves & needles	Gold	INAA	Stott (1996) unpub.
Western Australia	*Eremophila & Melaleuca & Eucalyptus*	Leaves & stems	Gold	INAA ICP-MS and graphite furnace AAS	Lintern *et al. (1997)*
New South Wales	*Callitris columellaris or Callitris glauca*	Needles	Lead-Zinc-Arsenic	INAA AAS	Cohen *et al. (1998)*
Victoria	*Acacia melanoxylon, Cassinia aculeta & Eucalyptus ssp.*	Outer bark, twigs & leaves	Ballarat East goldfields	INAA Graphite furnace AAS	Arne *et al. (1999)*
New Zealand	*Quintinia acutifolia, Nothofagus fusca, N. menziesii, Olearia rani, Quintinia acutifolia, Schefflera diditata, Triodia pugens & Weinmannia racemosa*	Leaves	Cu, Pb, Zn, U, Ni	AAS	Timperley *et al.* (1970)
New Zealand	*Beilschmiedia tawa, Myrsine salicina, Nothofagus ssp. Olearia rani, Quintinia acutifolia & Weinmannia racemosa*	Leaves & twigs	The significance of biogeochemical parameters such as: Biological absorption coefficient (BAC); Relative absorption coefficient (RAC); Acropetal coefficient (AC); Temporal absorption coefficient (TAC) and Mobile element absorption coefficient (MAC).	AAS & Emission spectrographic	Brooks (1973)

1.2.3 Some key biogeochemical factors to be constrained for applications in mineral exploration programs

A major objective of using plants in a biogeochemical approach for mineral exploration is to use the chemical analyses of plant tissue to represent the geochemistry of the underlying geology. Ideally, geological substrates with an econically significant enrichment in commodity elements should be expressed by elevated or distinctive biogeochemical expressions in plants that colonise such substrates. Complications, however, can arise because

plant biogeochemical characteristics may not only be controlled by substrate. Some of the other important variables that may potentially influence plant biogeochemistry include:

- species characteristics;
- organ differentiation;
- seasonality;
- detrital inputs;
- landscape setting; and,
- sampling contamination.

Species Characteristics

Plants are a significant component of the regolith and landscapes across most terrestrial settings (Hill, 2003). Element concentrations in plants largely depend on: (i) the species-specific uptake and demand; (ii) the availability of an element; and, (iii) the allocation of elements to various plant organs (Ernst, 1995). The *Eucalyptus* genus dominates most of the Australian landscape, with the exception of the drier interior regions, where they can be localised within and along the margins of ephemeral streams. The *Eucalyptus* genus is diverse, including some 800 species (Brooker & Kleinig, 2001), and are part of the *Myrtaceae* family. *Eucalyptus* plants can be either low shrubs, such as the mallees (up to 10m in height), or a very large tree such as the mountain ash (*Eucalyptus regnans*), which grows up to between 70-120 m.

Different plant species have different biogeochemical characteristics; however, all plants require a range of macro- and micro-nutrients for their life cycle (Salisbury & Ross, 1992). The bioavailability of some metals, however, can be restricted due to constraints such as element solubility, binding properties with soil particles, and antagonistic and synergistic interactions (Dunn, *et al*., 1992). The majority of element uptake is via the root systems. This is made possible due to characteristics of most root tips, which are typically weakly charged and slightly acidic, resulting in the exchange of H^+ for metals such as Cu, Zn and Ni at the colloidal interface (Keller & Fredrickson, 1952). Simple diffusion of elements is also important. Incorporated elements may then translocate to various plants organs (e.g. leaves, twigs, fruit, bark and roots) due to their different physiological roles in the plant (Brooks, 1972). This differential translocation of elements will produce a chemical heterogeneity within the plant and their eventual destination is important in the decision as to which organ is to be sampled.

Plant biogeochemistry largely reflects differences in biogeochemical process as well as different plant structures, such as root morphology and penetration depth, and therefore different access to underlying geological substrates. Many previous Australian biogeochemical studies have not made the distinction and identification of sampling media beyond the genus level. For example, Lintern *et al*. (2007) records biogeochemical results from Barns in the central Gawler Craton, South Australia as from a collective mixture from the *Eucalyptus* genus, including *E. incrassata* and *E. socialis*. An earlier study from a nearby study site with an equivalent vegetation assemblage showed these species to have significantly different trace metal biogeochemical characteristics (Mayo & Hill, 2005; Mayo, 2005). Similarly, a recent study by Cohen *et al*, (1999) only recorded plant types sampled to the genus level, such as *Acacia*. The lack of species recognition severely limits the accountability and viability of these studies.

A characteristic of the river red gum is the development of a deep taproot system. The dense surface roots of a mature river red gum extend at least 20 m horizontally (Dexter, 1953) and

greater than 10m vertically (Davies, 1953). Field observations from western New South Wales indicate that even these figures are conservative. The horizontal and verticle extents suggested by Dexter (1953) and Davies (1953) infer that river red gums can have a biogeochemical sampling area > than 4000m^3 with potential for element uptake via their root system from the adjacent stream sediments, the shallow ground water aquifers within the alluvial sediments; and, buried/concealed bedrock or saprolite.

Many of the characteristics of river red gums are therefore consistent with the ideal criteria required of a biogeochemical sampling media as outlined by Dunn, *et al*., (1992) and Hill (2002). These include:

- an easy to identify plant species;
- a locally dominant and widespread distribution;
- a tendency to colonise areas of transported regolith cover (where bedrock related information is readily available);
- an extensive root system that may penetrate transported cover and possibly also provide a homogenised expression of a heterogeneous transported cover;
- an ability to retain many plant organs throughout the year; and,
- large, smooth and waxy leaves that may tend to shed detrital surface contaminats.

Organ differentiation

The uptake of elements by plants may be active or passive (Ernst, 1990). Each plant organ has a different capacity to store metals and, therefore, comparing the same plant tissue is the only way to draw valid conclusions (Hill, 2004). In general, the most recently absorbed essential and trace elements are translocted to the youngest parts of the plant/tree (Carlisle and Cleveland, 1958), resulting in these organs having the highest concentration of elements. In constrast, a study by Warren and Delavault (1949) in British Columbia established that Cu and Zn concentrations in fruit, needles, buds and leaves had greater element variations than twigs. In conclusion Warren and Delavault (1949) recommended the sampling of 1 and 3 year old twigs with a girth ranging from one-eighth to one-fourth of an inch. As for eucalypts, the translocation and distribution of nutrients are related to differences in their physiological function and their relative mobility within the tree (Grove *et al*., 1996). The lack of information on the distribution of both essential and trace elements throughout the *E. camaldulensis* demonstrates the critical importance in characterising their major organs (i.e. leaves, twigs, bark, buds and fruit), in the preliminary stages of a biogeochemical survey, such as will be undertaken in this study.

Plant age

There have been very few studies that have considered the impact of plant age and elemental composition (England and Attiwill, 2008). Typically most previous studies do not record the age of the plants sampled and it is assumed (or hoped) that the plant ages within a single survey are comparable. Plant age is likely to be a major variable controlling the element composition of plants (Ernst, 1995), in particular annuals. In contrast, studies by Laclau *et al.*, (2003) demonstrate that nutrient requirements of *Eucalyptus alba* Reinw. Ex Blume increased sharply during the early growth period to reach a maximium around the 2 year age, and from this age onwards requirements for N, P and Ca remained stable, with a slight decrase in K and Mg. Additionally, for perennial trees, once the plant reaches an age of 2-3years, the uptake and translocation of both essential and trace elements from the soil becomes constant and the requirements of new growth causes only a gradual increase in element concentration (Carlisle and Cleveland, 1958). Further to this, plants have evolved so that elements that are scarce can be re-translocated from senescent tissues (e.g. leaves). Sampling leaves of the same

physiological age in a given season for elememental composition should therefore reduce the likelihood of effects induced solely on developmental nutritional requirements.

This study will only take samples from plants of apparent (morphological) equivalent age. This will include the targetting of large mature trees, which are estimated to be >100 years old.

Seasonality

Studies have shown that throughout the year, the elemental composition of living plant tissue can vary as the plant grows (Dunn, 2007). For the most part, these biogeochemical studies have been undertaken in cold to temperate climates, and have demonstrated that there is a pronounced seasonal element variation within the plants sampled (Dunn 1983, 1984, Stednick *et al.* 1987). Dunn (2007) advises that biogeochemical sampling should be undertaken in as short a time frame as possible (2-3 weeks) and preferably through the growing season. Equivalent studies from semi-arid to arid terrains are more limited. One study undertaken by Huenneke *et al.* (2001) in the Chihuahuan Desert of North America, however, proposes that vegetation in semi-arid terrains displays highly variable temporal chemical changes. These may be associated with the following annual growth cycle:

(i) winter - most species are dormant;
(ii) spring - reproduction has begun;
(iii) summer - summer rains give rise to peak reproduction activity; and,
(iv) autumn - peak biomass.

The parameters established by Huenneke *et al*. (2001) imply that the greatest concentration of trace metals is most likely to occur in summer for semi-arid arid terrains. During the summer period increases in temperature, available rainfall and daylight length will enhance plant growth and many reproductive processes. Theoretically these factors will contribute to an increase in evapotranspiration rates, resulting in a greater movement of elements transported throughout the xylem and allocated to specific sites depending on their physiological role and the increase in cellular activity during the peak period of reproduction (Garland 1981). Very little is known about the amount and variation of the essential and non-essential elements of eucalypts (Bhimaya and Kaul, 1966). A challenge for Australian semi-arid and arid areas, however, is that rainfall patterns are irregular, especially near the summer-dominated to winter-dominated rainfall transition zone, such as occurs in western NSW (Hill 2004). The general understanding gained so far suggests that in semi-arid and arid regions of Australia, there are no distinct and regular seasonal growing periods because of the irregular rainfall patterns that are a major control on plant growth (Hill 2002).

This study will incorporate an account of seasonal variations in the river red gum results from a range of sample sites in the study area.

Detrital inputs

One of the most significant types of contamination in biogeochemical surveys, particularly in semi-arid and arid regions, is the physical addition to samples from their substrate, aeolian particles and soil splash (Hill, 2002). Detrital inputs such as soil and aeolian particles can become lodged in plant tissue and the removal of these particles can be diffucult. Dunn, (2008) recognizes that a minimal distance of 100 m from busy and dusty trails and 50 m from tacks with very little traffic is necessary for the prevention of false anomalies and in the reduction of detrital contamination to samples. The identification of detrital contaminates can be achieved by the use of soil tracer elements such as Al, Ti, Pu, Sc, Zr, Fe and Si (Bargagli,

1998; Hill, 2002). Generally these elements have a greater affinity for inorganic soil-regolith particles rather than their potential to be bioavailable for incorporation into plant tissues from root uptake. Other sources of detrital contamination may include: ore smelting; nearby metal contrustions; animal faeces; films secreted from insects and wind blown fertlisers (Hill, 2002). This study will examine the significance of detrital inputs to plant samples by scrutinising the soil tracer element abundances within results as well as conducting a detailed scanning elecron microscope (SEM) study of leaf samples.

Landscape Setting

The landscape setting is one of the most important influences on the distribution of elements within the regolith substrate (Hill, 2004; Hill *et al*., 2005), and therefore potentially on the biogeochemical characteristics of plants. Broadly, regolith profiles across a given area are the result of numerous eposides of weathering, erosion and deposition over an extended period (Fabris *et al*., 2008). Figure 1.3 illustrates some major regolith materials from semi-arid landscapes of the stiudy area and their typical landscape settings.

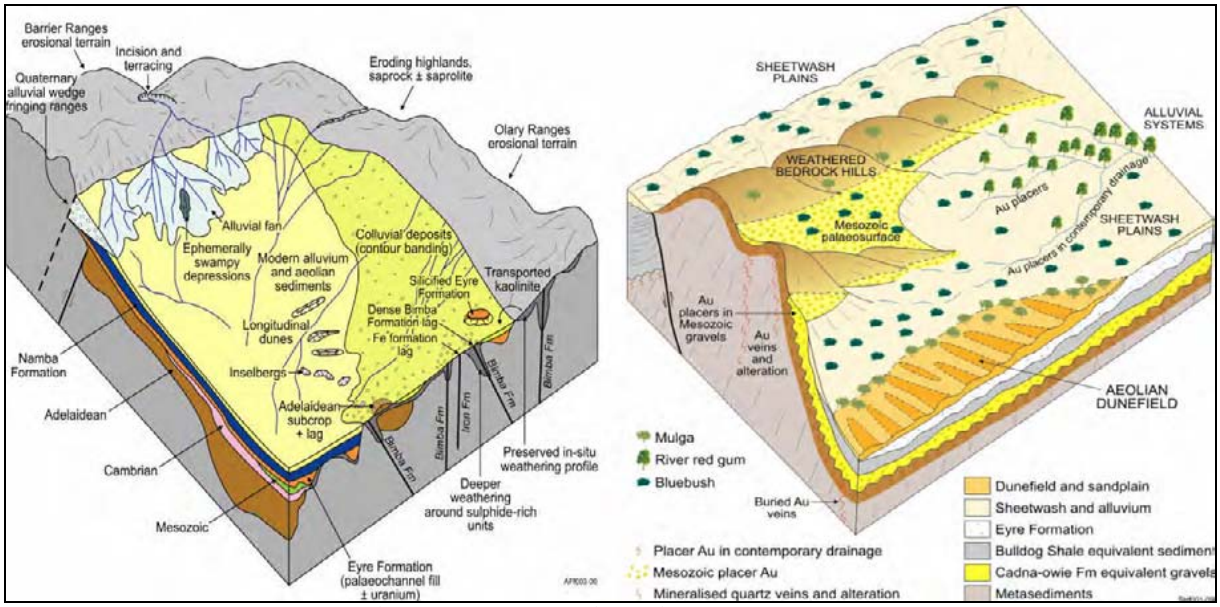


Figure 1.3: Cartoons representing on the left, the main regolith-landforms of the Olary and southern Barrier Ranges (Broken Hill) (Fabris *et al*., 2008), and on the right for Au mineralisation in the Tibooburra-Milparinks region (Hill *et al*.,, 2008).

The regolith can be broadly defined as 'everything between fresh rock and fresh air'. For mineral exploration purposes the regolith can be divided in to three broad types of material (Fabris *et al*., 2008 and Hill *et al*., 2008):

- in situ regolith;
- transported regolith (sediments); and,
- indurated regolith (duricrusts and pans);

These materials include an assortment of rock-derived, extraneous (e.g. introduced salts) and biogenic components (roots and decaying vegetation). The following Table 1.3 outlines the characteristics that define the three broad regolith types and the suggested mineral exploration approaches for areas with these materials.

Table 1.3 Outlines the characteristics of the three types of regolith materials their, landform types and mineral exploration reccomendations taken from (Hill *et a*l., 2008; and Fabris *et al*., 2008).

Regolith materials	Landform	Mineral exploration reccomendations
in situ exposed and near surface bedrock that have undergone some degree of weathering. Indicators of *in situ* regolith include; preservation of primary bedrock fabrics, textures and structures. Surface lags contain an increase in angular quartz vein clasts. Colonistaion by plants such as mulga (*Acacia aneura*), whitewood (*Atalaya hemiglauca*) and rock sida (*Sida petrofolia*).	Rises, plateaux, erosional rises, escarpments, pediments and rises.	Biogeochemistry (sampling of the most dominant plant in the chosen region), ferruginous lags and ferruginous duricrusts.
Transported regolith (sediments) consists of an assortment of marine, alluvial, aeolian and lacustrine sediments, consisting of sand, silt and clays.	Depositional plains and playa lakes.	Biogeochemistry (sampling of the most dominant plant in the chosen region), stream sediments/carbonate sampling which may give a broad chemical signature of the catchment.
Indurated regolith (duricrusts and pans) are materials that have hardened or been cemented by groundwater or near surface pedogenic process.	Topographic inversion (atop ridges and as mesa cappinngs on erosional plains).	Biogeochemistry (sampling of the most dominant plant in the chosen region), and lithic lags sampleing may provide a broad chemical signature of the catchment.

The characterisation of regolith materials and their landform context, shown in a regolith-landform map provide valuable information and framework for mineral exploration programs in regolith-dominated terrains. This information should also provide an important context for interpreting biogeochemical results, in particular for distinguishing between controls from bedrock substrates rather than landscape setting. Surprisingly few previous biogeochemical studies have incorporated a regolith-landform map or context into the interpretation of results. This study will be one of the first to try and address regolith-landform controls on plant biogeochemistry, largely by presenting the spatial results from study sites relative to a regolith-landform map.

Sampling Contamination

Biogeochemical samples maybe contaminated by a number of sources such as: sunscreen; jewwllery and field equipment. The consistency and accountability of minimal sampling contamination is a major factor that underpins the success and reliability of a biogeochemical sampling program. Typically the elemental abundances measured in these programs are less than the parts per million and parts per billion scale. Therefore minor contamination can have a significant impact on results. Although any form of contamination is unlikely to be totally excluded the object is for it to be minimised and at worst consistently accountable.

The significance of this requires that considerable effort has been invested in establishing a robust and consistent sampling approach for this study. This incorporates some features from previous work but in essence is a tailor-made approach suitable for river red gum trees. The sampling approach established in this study should form the basis of other river red gum sampling programs in Australia, and as such the details of this sampling method are outlined in the methodology chapter of this thesis, but are also summarised in the implications of this study discussion.

1.3 GEOLOGICAL SIGNIFICANCE AND SETTING OF THE CURNAMONA PROVINCE AND ADJACENT AREAS IN SOUTHEAST INLAND AUSTRALIA

The Curnamona Province provides a geologically defined geographical basis for this study. It is a world renowned, highly perspective mineral terrain, of considerable geological research interest both nationally and internationally. The world class Broken Hill Pb-Zn-Ag deposit and a wide range of other mineral occurrences are included in the Province. A majority of the bedrock in the Province, however, is concealed by transported regolith, which continues to restrict the ability of many mineral exploration techniques to be successful. The development of an understanding of river red gum biogeochemistry in this region is therefore of great economic importance.

1.3.1 Regional Geological Setting

The Curnamona Province is an 'oval-shaped' area of approximately 100 000 km^2 (Teale & Flint, 1993) that extends across central eastern South Australia and western New South Wales (Figure 1.4). The geological units consist of deformed, late-Palaeoproterozoic metasedimentary and metavolcanic rocks (Willyama Supergroup) with some meta-intrusives and early Mesoproterozoic volcanics, sediments and granitic intrusives. Exposures occur as a series of inliers within ‘corridors’ of Neoproterozoic Adelaidean rock, which are also extensively concealed by Mesozoic and Cainozoic sediments. Major exposures of bedrock in the Curnamona Province are in eastern South Australia (along the ridges of the ‘Olary Spur’) and in western New South Wales (within the Barrier Ranges near Broken Hill). Possible extensions of the Curnamona Province occur to the northwest in the Mount Babbage and Mount Painter Inlier of the northern Flinders Ranges (Campana & King, 1958; Drexel & Preiss, 1995; and Robertson *et al*., 1998).

Figure 1.4: Geology of the Oval shaped Proterozoic Curnamona Province that extends across central eastern South Australia into western New South Wales after (Leyh & Conor, 2000).

The oldest lithological units in the Province are from the Willyama Supergroup. Willis *et al*. (1983) identified the sequence to generally fine upwards, with an increase in compositional maturity of the sediments from feldspathic metasedimentary composite gneisses and migmatites to pelitic metasediments. Rocks of the Willyama Inliers include the Olary Domain and Broken Hill Domain (Table 1.4). The two domains are mostly sub-divided based on their lithological and geophysical characteristics (Robertson *et al*., 1998). Summaries of these differences are outlined in (Table 1.4).

Table 1.4: Comparisons of the geological features of the Willyama Inliers between the Olary Domain and Broken Hill Domain (after Willis *et al*., 1983; Yates & Randell, 1994; and, Robertson *et al*., 1998).

	Olary Domain	**Broken Hill Domain**
Willyama Supergroup thickness	~5 km	4.5 and 13 km
Main lithologies	Quartzo-felspathic gneiss to albatites and calc-silicates	Quartzo-feldspathic rocks, hornblende bearing amphibolites, pelitic → psammopelitic metasediments
Geological setting	Shallow marine interspersed with non-marine playa lakes	Quartzo-felspathic gneiss to calcsilicate-rich and pelites
General metamorphic grade	Amphibolite to greenschist	Granulite to amphibolite (south) and greenschist (north)
Prominent mineralisation	Cu-Au, minor U	Pb-Zn-Ag
Geophysical properties	Gravity low	Gravity high
Geochronology	Palaeoproterozoic	

Late Proterozoic (Adelaidean) metamorphosed sediments and volcanics unconformably overlie the Willyama Supergroup in both domains (Stevens, 1986, Stevens & Burton, 1998). Sedimentation of the Adelaide Geosyncline (Preiss, 1987; Preiss, 1990; Preiss *et al*., 1993 and Robertson *et al*., 1998) commenced at the start of the Willouran times (approximately 850 Ma), with the deposition of mature quartzose sands and localised conglomerates. These rocks consist of:

- Willouran series: medium to coarse-grained quartzite and sandstone interbedded with dark finely laminated siltstones. The depositional environment is interpreted as either floodplain or tidal (Forbes & Preiss, 1987);
- Torrensian series: coarse conglomerate, overlain by alternating argillaceous and dolomitic beds, with minor lenses of sedimentary magnesite. The depositional environments are interpreted as a fluvial to shallow marine, followed by lagoonal to shallow marine and a deeper marine shelf (Robertson *et al.*,1998);
- Sturtian series: irregular alternation of boulder tillite, tillitic siltstones, quartzites, shales and dolomites. The depositional environment is interpreted as a period of deglaciation with extensive marine transgression (Robertson *et al.*,1998); and,
- Marinoan series: laminated dolomite, quartzite and siltstones with interbedded dolomite, bedded siltstones and fine sandstone, flaggy limestone, siltstone, shale and sedimentary breccias. The depositional environment is interpreted as marginal to deep marine (Forbes & Preiss, 1987).

The Adelaidean units covering the Curnamona Province in South Australia (Olary Domain) and western New South Wales (Broken Hill Domain) locally have different stratigraphic names, but are considered equivalent (Figure 1.5).

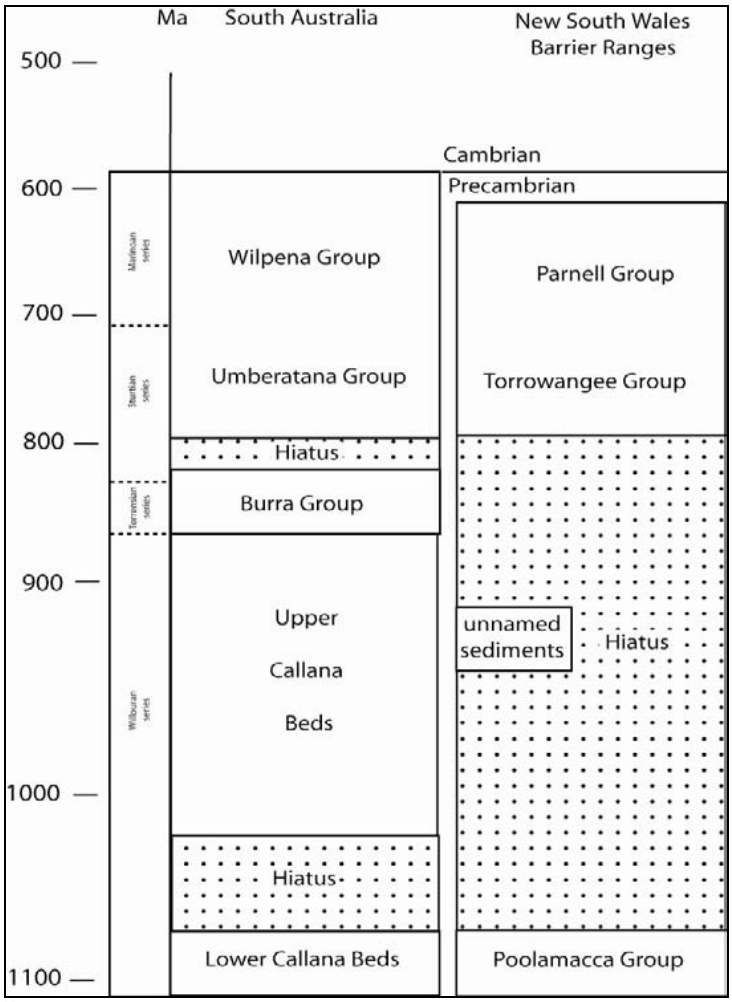


Figure 1.5: Outlines the differences in stratigraphical names and the approximate correlations between South Australia and New South Wales, after Stevens (1986) and Willis *et al*. (1983).

Sedimentation in the Adelaide Geosyncline was reduced towards the end of the Cambrian with the onset of the Delamerian Orogeny (Preiss, 1995). A major disconformity separates the Adelaidean from overlying Cambrian rocks (Robertson *et al*., 1998) within the Olary Domain. The Anabam Granite followed this sedimentation hiatus, within the Ordovician (Preiss, 1995).

To the east of the Broken Hill Domain are the sediments within the Palaeozoic Koonenberry Belt and include the Gnalta Group (Early Cambrian) and Mootwingee Group (Middle Ordovician) sediments. The sediments consist of fossiliferous limestone, quartzite and shale horizons indicative of a continuous shallow water environment (Rose & Brunker, 1968). In northwestern New South Wales the Silurian, is interpreted as a time of depositional hiatus; however Rose *et al*. (1967), and Rose & Brunker (1968) suggest that varying sized bodies of granite (e.g. Tibooburra Granodiorite) intruded at this time.

In the Middle to Upper Devonian an extensive body of shallow water covered much of northwestern New South Wales, which saw the deposition of dominantly sandstones with minor conglomerates and siltstones of the Snake Cave Sandstone, Mulga Downs Group and the Coco Range Beds (Rose & Brunker, 1968; Rose *et al.*, 1967).

These sediments unconformably overlie the Ordovician and Lower Devonian units. Succeeding this was the deposition mostly quartzose sandstones of the Nundooka Sandstone and Ravendale Formation, during the Devonian to Carboniferous (Rose & Brunker, 1968).

During the late Palaeozoic (Permian) an extensive glacial ice sheet covered most of southern Australia (Ollier, 1986). As the glacial ice sheet retreated, a blanket of unconsolidated debris, covered much of the landscape (Flint et al., 1980; Brakel, 1993; Hill, 1999).

In the Mesozoic a large area of continental and shallow marine deposits associated with the Eromanga Basin extended across many parts of the region (Rose & Brunker, 1968). The sediments included quartzose gravels and sands with minor silts, which are part of the late Jurassic to Early Cretaceous sediments locally equivalent to the Algebuckina Sandstone and the Cadna-owie Formation. In the Tibooburra region these sediments have been collectively referred to as the 'Gum Vale Formation' (Morton, 1982). Directly overlying this are shallow marine fine sands, silts and clays of the Rolling Downs Group (Kenny, 1934), which in the Tibooburra region include fine-grained sandstones, siltstones and claystones of the locally defined Wittabrenna Beds (Morton, 1982), and are regionally equivalent to the Bulldog Shale of the Maree Sub-group (Kreig *et al.*, 1995). Sediments associated with the Mesozoic marine regression are poorly documented from the study area, but may include fluvial to lacustrine mid to late Cretaceous sediments of the Winton Formation near Tibooburra.

The Olary Domain and the Broken Hill Domain are also overlain and flanked by Cainozoic sediments. In the early Cainozoic, tectonic warping and faulting in the south led to the development of the Murray Basin, which accommodated the deposition of terrestrial alluvial, aeolian, colluvial and lacustrine sediments as well as shallow marine to estuarine clay and limestone on the southern margins of the Curnamona Province (Brunker *et al.*, 1967; Forbes, 1991). In the north and northwest of the Curnamona Province, largely overlying the Eromanga Basin sediments, Cainozoic fluvial, lacustrine and aeolian sediments from the Lake Eyre Basin form the youngest transported cover (Callen *et al*., 1995; Alley, 1998).

1.3.2 Mineralisation Potential across the Curnamona Province

The region hosts a wide range of mineralisation types such as iron oxide-copper-gold (IOCG) deposits; Zn-Pb-Ag (Broken Hill and Mt Isa-McAuthur Basin style) deposits, vein and placer Au; and bedrock hosted (e.g. hematite breccia and vein) and sedimentary hosted U. The region is best known for the enormous Broken Hill (Pb-Zn-Ag) ore body. Areas such as the Tibooburra-Milparinka district and the Koonenberry Belt, flanking the Curnamona Province, also host minor mineralisation occurrences and small historical mining operations. The Milparinka-Tibooburra district in the north has Au occurrences in both quartz veins and in alluvial units such as in basal conglomerates of the Mesozoic sequence and reworked Cainozoic deposits. Throughout the Koonenberry Belt smaller occurrences of Au have been mainly obtained from the base of the Mesozoic cover (e.g. Williams Peak) and as younger alluvial gold (Mills & Hicks, 2001). There are also records of Cu, Zn, Ag and Au bearing massive sulphide mineralisation within the Koonenberry Belt, such as at Grasmere (Buckley, 2000).

The search for minerals in the Olary Domain dates back to the 1860s, and the discovery of Au in the Waukaringa district in 1872 (Campana & King, 1958). The largest mine in the Olary region was Radium Hill (Forbes, 1991), which was discovered in 1906 and worked for veins of U-bearing minerals, such as davidite, sporadically between 1911-1964.

The regolith extends across about 90% of the region with most known mineralisation restricted to the 10% of exposed bedrock. The limited effective exploration in regolith-dominated terrains across the Curnamona Province and adjacent regions has therefore left these areas under-explored and therefore a frontier for mineral exploration.

1.4 THE AIM OF THIS STUDY

The main aim of this study is to determine the potential application of *E. camaldulensis* as a mineral exploration sampling medium. In mineral exploration programs, the main objective is to isolate the contributions from geological substrates to plant biogeochemistry, which is usually done by constraining or minimising the biogeochemical expression from other factors. In order to account for a range of controlling factors, this research implements a multidisciplinary approach, utilising techniques derived from botany, biogeochemistry, geology, geochemistry, geomorphology and regolith geology. It involves two main components representing study at different scales:

1. study at the scale of individual trees. This involves the characterisation of internal biogeochemical characteristics within trees between plant organs and variations in canopy aspect, as well as temporal variation. Comparison is also made between plant chemistry and substrate (stream sediment) geochemistry for individual trees; and,
2. the study of populations of trees within the landscape on the scale of catchment headwaters. This enables the identification of landscape and environmental factors that may influence plant biogeochemistry, such as regolith-landform and the underlying geological setting.

Once the biogeochemical expression and chemical interactions between the underlying bedrock and the surficial plant organs are better characterised and understood, the application of river red gum biogeochemistry for mineral exploration programs can be better assessed. In summary, a general research hypothesis to be tested is that:

"*Eucalyptus camaldulensis* have the ability to delineate areas of buried mineralisation from surrounding unmineralised areas".

1.5 OBJECTIVES OF STUDY

The specific objective of this study is to characterise the individual tree and regional biogeochemical attributes of *E. camaldulensis* in the Curnamona Province and adjacent areas in SE central Australia. These results are then used to constrain and interpret some of the environmental controls on *E. camaldulensis* biogeochemistry with the intention of isolating geological substrate controls on biogeochemistry. This will enable an assessment to be made for the applications and implications for mineral exploration programs.

At the scale of individual trees the detailed study involves the characterisation of 6 individual tress at key sites across the study area, including:

1. characterising and comparing the biogeochemistry of *E. camaldulensis* major organs (i.e. leaves, twigs, bark, fruit, buds and bark);
2. defining spatial biogeochemical variations/heterogeneity within individual trees by sampling around the circumference of the accessible tree canopy; and,
3. defining temporal variations and trying to account for these relative to environmental variables.

The results and interpretations from this detailed phase of the research are then expanded from individual trees, to catchment-scale biogeochemical surveys at Tibooburra (Racecourse Creek) and the Barrier-Pinnacles mine (Pine Creek). At these two catchment headwater-scale sites the study aims to:

- characterise the spatial biogeochemical variations between adjacent trees along the main catchment channels.

1.6 THESIS STRUCTURE

Following this introduction to this thesis study (Chapter 1), an outline of the study methodology is given in Chapter 2. This includes the information pertaining to the development of a robust sampling, sample preparation and analytical approached used in this study and recommended for further use in mineral exploration programs.

The results from the detailed individual tree studies are the given in Chapter 3. Six individual trees from different geological settings across the study region were chosen as part of this study. This includes: a characterisation and comparison of plant organ biogeochemistry (Aim 1); definition of spatial heterogeneity within the tree canopy (Aim 2); and, results and interpretation from multiple sampling of trees over a two year time period to account for temporal variations in biogeochemistry (Aim 3).

The landscape-scale study or river red gum populations along headwater channels of stream catchments (Aim 4) are given in Chapter 4. This provides results that show the important environmental factors that influence biogeochemical characteristics across the landscape (Aim 5). An ability developed in this chapter to use the biogeochemical results to provide a penetrative geochemical expression of buried mineralisation, such as is typically required for mineral exploration applications (Aim 6), culminated in a major discovery of mineralised lodes buried by transported regolith crossing Pine Creek, near the Pinnacles Mine. This is an important "proof-of-concept" for the approach developed in this study, and the mineral discovery made from using this technique, where other approaches failed for over 100 years of exploration, provides highly credible endorsement for the application of this research.

CHAPTER 2

METHODS

This project employs a multidisciplinary range of established and innovative methods derived from botany, biogeochemistry, geochemistry and regolith-landform mapping, to investigate the potential biogeochemical application of *E. camaldulensis* as a mineral exploration sampling medium throughout the regolith-dominated Curnamona Province and adjacent regions. This chapter outlines the research methods used in this study.

2.1 MAPPING METHODS

2.1.1 Aerial photographic interpretation

The initial characterisation of regolith-landform units across the Tibooburra-Racecourse Creek and Barrier Pinnacles mine - Pine Creek (Broken Hill) field sites, was based on stereoscopic interpretation and film annotation of 1:50 000 aerial photographs (Tibooburra, SH540707; NSW4483 (M2179) run 7 07-07-99, 36-47 and Thackaringa, S1540302; NSW4283 (M2033) run 2 07-09-95, 01-12). The aerial photographs were scanned at a high resolution (1200 dpi), georeferenced (ERMapper 3.2), and printed at 1:10 000 scale. They conformed to the World Geodetic System (WGS84), in the Universal Transverse Mercator (UTM) projection, Zone 54 South.

The application of aerial photographs for the initial interpretation of regolith-landforms is based on their expression of landform variations through stereoscopic projection and the colour, tonal and pattern differences that can relate to regolith material. This broadly reveals areas of bedrock exposure (*in-situ*), erosion, and deposition. This enables the indirect mapping (remotely sensed) of the field area resulting in the production of a preliminary regolith map (Hill, 1995). This preliminary regolith map is then verified by field characterisation, recording attributes for each regolith-landform unit (RLU).

2.1.2 Fieldwork

Initial fieldwork was undertaken during October 2003. One month was spent in the field with the time split between the two main field sites. During this time the preliminary regolith-landform map annotation and interpretations were scrutinized, including the position of unit polygon boundaries and field descriptions of RLUs. The following attributes were described for each field site:

1. GPS (global positioning system) Coordinates
    - Eastings and Northings, determined from a Garmin GPS 12, which is typically accurate to ± 15 horizontal meters. The datum used was WGS (World Geodetic System, 1984) in the Universal Transverse Mercator (UTM) projection;
2. Dominant landform expression

- topographic relief, slope gradient, aspect (facing) and dominant geomorphic processes;

3. Dominant regolith lithology
    - attributes of weathered bedrock (type and weathered features), transported regolith and indurated (secondary cementation) regolith materials. This includes colour, composition, grain size and morphology, sorting and estimated proportions of the different components;
4. Surficial features
    - material exposed across the land surface and overlying the dominant regolith geology, such as surface lag (e.g. colour composition, grain size and morphology, sorting and estimated proportions of the different components);
5. Minor attributes
    - attributes that are not dominant enough to form discrete mappable units at this scale (e.g. red-brown clays within the surface lag and deposited aeolian sands);
6. Vegetation
    - vegetation community type and dominant species; and,
7. Geohazards
    - These were mostly erosional hazards such as gullies, sheet erosion, wind and rabbit warrens.

In total 37 regolith-landform units at Tibooburra and 34 regolith-landform units at the Barrier Pinnacles mine were defined and mapped. This information aided in the compilation of the 1:10 000 regolith-landform maps for both Tibooburra (Figure 4.3) and Pinnacles (Figure 4.26).

2.1.3 Regolith-landform mapping scheme

Regolith-landform units identified at Tibooburra and the Barrier Pinnacles Mine were based on the mapping scheme developed by the Bureau of Mineral Resources (now Geoscience Australia) as outlined in the RTMAP BMR regolith database field book and users guide (Pain *et al*., 1991; Pain *et al.*, 2007) (Table 2.1). The scheme uses an alpha-numeric code to label the RLUs (Table 2.2), including, upper case letters to represent the dominant regolith materials, and lower case letters to denote the dominant landform expression. Subscript numbers, signify minor variations and modifiers between similar regolith-landform units. A more detailed description of the regolith, landform, surficial features, minor attributes, vegetation and erosion hazards is provided in the map legend.

Table 2.1: Typical regolith and landforms used in this study (derived from Pain *et al*., 1991).

NOTE:
This table is included on page 21 of the print copy of the thesis held in the University of Adelaide Library.

Table 2.2: A regolith and landform code arrangement for polygon labels (derived from Pain *et al*., 1991).

NOTE:
This table is included on page 21 of the print copy of the thesis held in the University of Adelaide Library.

2.1.4 Map production

The georectified 1:10 000 regolith-landform overlay draft maps and base maps were drum scanned at the Adelaide offices of Primary Industries and Resources South Australia (PIRSA). Both base maps were converted into ecw (Enhanced Compressed Wavelet) files while the regolith-landform overlay draft maps were converted into j-pegs (Joint Photographic Experts Group) files. Base and regolith-landform overlay files were opened in ArcInfo 8, and set at their projected World Geodetic System (WGS84) coordinates in the Universal Transverse Mercator (UTM) projection Zone 54 South. All polygons were drawn individually and labelled with their corresponding RLU codes.

2.2 VEGETATION AND STREAM SEDIMENT SAMPLING

2.2.1 Sample site recording

At *E. camaldulensis* sample sites, GPS coordinates were recorded, and brown paper sample bags labelled. Descriptive notes were made of the immediate surroundings, including:

- potential contaminants, particularly anthropogenic inputs (such as the distance from main roads);
- landform setting (e.g. upper catchment head waters or lower in the catchment);
- regolith materials (including if they are of heterogeneous or homogeneous composition);
- surface expression (e.g. erosional landforms, depositional landforms, fans, plains or depressions);
- other external factors that could influence the *E. camaldulensis*; and,
- other dominant vegetation species.

2.2.2 Plant organ sampling

It is usually practical and advisable to collect samples at about chest height form around the circumference of the tree. This ensures that the sampling comparably and adequately represents the element composition of the total tree (Allen, 1989; Dunn, 2007). Brooks (1972) reccomends at least sampling from several points around the circumference of the tree, due to the thought that root systems tend to only translocate elements to aerial parts on the same side of the tree. Therefore samples collected for this study were limited to those of similar age (fully open mature leaves) and between chest and head height from around the circumference of the canopy.

A reconnaissance trip in late March 2003 visited the Curnamona Province and adjacent regions (central E of SA and far NW of NSW), for the selection of six individual *E. camaldulensis* trees for detailed study. The location of the chosen *E. camaldulensis* was based on their proximity to different geological and mineralised settings, and the availability of a pre-existing regolith and geochemical framework. Sampling of the six individual *E. camaldulensis* trees was undertaken within each of the major seasons (autumn, winter, spring and summer) over the course of three years.

The canopies of the selected *E. camaldulensis* were sub-divided into N, NE, E, SE, S, SW, W, NW and N sectors. Samples of leaves, bark, twigs, fruit, buds and bark were taken at from these sectors (where available) from the six locations.

Immediately prior to sampling, potential leaf and twig samples were checked for their health and maturity to ensure that they were not carrying prominent fungal growth and faecal deposits. Recovery of twigs was achieved by using Teflon-coated clippers. The twigs were sampled along with the leaves by separating them by tearing leaves at their stalk base. This procedure allows for the further collection of fruit and flowering buds. A 5x10 cm block of the smooth and tough bark, was cut with a hatchet and then peeled away. Root sampling was variably successful depending largely upon the landscape setting of individual trees. For

samples within alluvial plains, the collection involved digging for the roots, while samples from erosive drainage channels typically had some roots exposed, and therefore allowed for simple collection with the aid of a small hatchet. In conjunction with the sampling of *E. camaldulensis* organs, 5 kg of stream sediments from the drip line of the canopy at the compass bearings of the eight sectors were also recovered. Stream sediments were sieved to the following size fractions: <75 μm and 75-300 μm.

Racecourse Creek (Tibooburra) and Barrier Pinnacles-Pine Creek (Broken Hill) sites were chosen for a more detailed survey. This involved sampling every *E. camaldulensis* with leaves available along a stretch of channel.

To minimise contamination and to ensure that a consistent sampling procedure was met all samples and site-recording details were performed by the author of this study.

2.2.3 Sample storage

Brown paper lunch bags were used for the storage and transport of the vegetation media. Brown paper lunch bags allow the sample to breath, reducing the rate of respiration that may lead to organic breakdown (Dunn. 2007). Bradfield & Bould (1963, cited in Allen, 1974) and Hill (2002; 2004) have suggested that plant organ storage in paper bags for up to 4 days will result in minimal changes to biogeochemical composition.

The method of storage that was employed in the study was:

- all samples collected were placed in individual brown paper bags (235 mm x 200 mm);
- the bags were sealed by folding over the top; and,
- samples were stored in a well-ventilated and dry area for the duration of the sampling period.

Stream sediments were stored in zip-lock plastic bags (258 mm x 328 mm) for transport to the laboratory.

2.2.4 Quality control

To reduce the potential for contamination during sampling, all jewellery and other metallic objects were removed, and hands were washed and air dried if sunscreen had been applied (hats and the shade of the trees provide temporary sunscreen while sampling). Non-powdered, latex gloves were worn on at least one of the sampling hands (the hand that came directly in contact with samples). Gloves were changed between samples to minimise the risk of cross-contamination between samples. The sampling of twigs required the use of Teflon-coated clippers. To minimise cross-contamination between samples, the clippers were cleaned with either distilled water or ethanol. In addition, several incisions were made into twigs from the tree to be sampled prior to cutting the samples, with the intention of pre-contaminating the clippers with the twig samples prior to their collection.

In order to evaluate the precision and accuracy of the analysis on the *E. camaldulensis* samples, field/blind duplicates were also inserted at approximately every 50th sample, along

with three International Analytical Standards every 30th sample, (See 'Chemical analysis,' for details of International Analytical Standards).

The method of preparation of the blind duplicates can be found in section 2.5 SAMPLE PREPARATION (pre-digestion) and for both the blind duplicates and the International Analytical Standards see section 2.6 SAMPLE PREPARATION (digestion) and assay), for Inductively Coulped Plasma Mass Spectrometry (ICP-MS) and X-Ray Fluorescence (XRF). The object here is to evaluate the analyses and and how representative they are, and thereby ascertain their:

- precision: reproducibility i.e. how close are a series of mesurements on the same sample to each other, and;
- accuracy: the agreement between the obtained result from a measurement and the true value.

2.3 CHEMICAL ANALYSIS

The level of analytical precision and accuracy of *E. camaldulensis* analyses was expressed through the analysis of duplicate *E. camaldulensis* samples, and the addition of: two types of National Bureau of Standards (NBS) samples - Pine Needles (Standard Reference Material, 1575) and Orchard Leaves (Standard Reference Material, 1571); and, one Community Bureau of Reference, Belgium (BCR) Wholemeal (certified reference material, 189). Table 2.3 outlines the precision and accuracy of the analytical method using Standard Reference Material. Table 2.4 shows the level of precision and accuracy among multiple measurements of the same sample. Duplicates in general returned concentration values (precision) between 80 – 100 % of each other, while the certified Standard Reference Materials returned concentration values (precision) between 32 – 100 %.

Table 2.3: Conformation of analytical precision and accuracy was determined by the use of *E. camaldulensis* replicates and NBS samples. A summary of the Analytical methods, detection limits, vertified values and recorded values obtained. Precision % = (SRM – value) / SRM x 100 %, where SRM is the standard reference material, with accuracy being the Standard deviation of the data set. Below D/L = below detection limit.

Elements Certified	**Method**	**Detection limit (ppm)**	**Certified values (ppm)**	**Recorded (SRM: Orchard leaves)**	**Precision %**	**Accuracy: Mean (+/- Standard deviation)**
Be	ICP-MS	0.1	0.027 ± 0.010	Below D/L	N/A	N/A
As	ICP-MS	0.5	10 ± 2	7.8 - 9	78 -90	8.74 (+/- 0.51)
Mo	ICP-MS	0.01	0.3 ± 0.1	0.1 – 0.2	34 - 67	0.18 (+/- 0.04)
Cd	ICP-MS	0.05	0.11± 0.01	Below D/L	N/A	N/A
Sb	ICP-MS	0.01	2.9 ± 0.3	1.8 – 2.2	63 - 76	2.1 (+/- 0.18)
Th	ICP-MS	0.10	0.064 ± 0.006	Below D/L	N/A	N/A
U	ICP-MS	0.07	0.029 ± 0.005	Below D/L	N/A	N/A
Uncertified			**Uncertified Values (ppm)**			
Ga	ICP-MS	0.1	0.08	Below D/L	unknown	N/A
Ce	ICP-MS	0.02	0.04	0.78 – 0.87	0	0.82 (+/- 0.04)
Bi	ICP-MS	0.01	0.1	Below D/L	unknown	N/A
Element Certified	**Method**	**Detection limit (ppm)**	**Certified values (ppm)**	**Recorded (SRM: Pine Needles)**	**Precision %**	
As	ICP-MS	0.5	0.21 ± 0.04	Below D/L	unknown	N/A
Th	ICP-MS	0.10	0.037 ± 0.003	Below D/L	unknown	N/A
U	ICP-MS	0.07	0.020 ± 0.004	Below D/L	unknown	N/A
Uncertified			**Uncertified Values (ppm)**			
Ce	ICP-MS	0.02	0.04	0.22 – 0.26	0	0.24 (+/- 0.01)

Cd	ICP-MS	0.05	<0.05	0.16 – 0.23	32 – 64	0.17 (+/- 0.02)
Sb	ICP-MS	0.01	0.2	0.1 – 0.2	50 – 100	0.16 (+/- 0.05)
La	ICP-MS	0.02	0.2	0.11 – 0.12	55 - 60	0.12 (+/-0.004)
Eu	ICP-MS	0.50	0.006	Below D/L	unknown	N/A

Element Certified	**Method**	**Detection limit (ppm)**	**Certified values (ppm)**	**Recorded (SRM: Wholemeal flour)**	**Precision %**
Cd	ICP-MS	0.05	7.13	Below D/L	unknown
Uncertified			**Uncertified Values (ppm)**		
As	ICP-MS	0.5	1.8	Below D/L	unknown

Table 2.4: The level of precision and accuracy among multiple measurements of the *E. camaldulensis*. The Analytical method used, detection limit and recorded triplicate values. Precision % = (maximum - minimum) / maximum x 100 %, with accuracy being the Standard deviation of the data set.

Element (species)	Detection limit (ppm)	Method	Precision %	Accuracy: Mean (+/- Standard deviation)
Be	0.1	ICP-MS	Below D/L	N/A
Na_2O	20	XRF	84 - 96	0.02 (+/- 0.01)
MgO	40	XRF	89 - 97	(0.2) +/- 0.01
Al_2O_3	10	XRF	81 - 90	440 (+/- 22)
SiO_2	60	XRF	91 - 93	0.22 (+/- 0.01)
P_2O_5	10	XRF	89 - 98	0.11 (+/- 0.01)
SO_3	10	XRF	92 - 99	0.08 (+/- 0.004)
Cl	5	XRF	97 - 98	5462 (+/- 59)
K_2O	20	XRF	98 - 99	0.89 (+/- 0.01)
CaO	20	XRF	95 - 97	0.91 (+/- 0.01)
MnO	10	XRF	97 - 99	146 (+/- 2.3)
Fe_2O_3	20	XRF	81 - 92	123 (+/- 13)
Ni	2	XRF	Below D/L	N/A
Cu	0.8	XRF	100	4 (+/- 0)
Zn	0.5	XRF	100	30 (+/- 0.58)
Ga	0.2	ICP-MS	Below D/L	N/A
Ge	0.1	ICP-MS	Below D/L	N/A
As	0.5	ICP-MS	Below D/L	N/A
Rb	1	XRF	100	1.6 (+/- 0)
Sr	0.5	XRF	96 - 98	44 (+/- 0.68)
Y	0.5	ICP-MS	Below D/L	N/A
Zr	1	XRF	Below D/L	N/A
Nb	0.1	ICP-MS	Below D/L	N/A
Mo	0.1	ICP-MS	Below D/L	N/A
Ag	0.01	ICP-MS	25 - 100	0.03 (+/- 0.01)
Cd	0.1	ICP-MS	82 - 100	0.15 (+/- 0.02)
Sn	0.5	ICP-MS	Below D/L	N/A
Sb	0.1	ICP-MS	Below D/L	N/A
Cs	0.01	ICP-MS	100	0.01 (+/-0)
Ba	10	XRF	81 - 100	19 (+/- 2)
La	0.02	ICP-MS	54 - 100	0.1 (+/- 0.02)
Ce	0.1	ICP-MS	66 - 100	0.22 (+/- 0.04)
Pr	0.01	ICP-MS	67 - 100	0.02 (+/- 0.01)
Nd	0.01	ICP-MS	73 - 86	0.1 (+/- 0.01)
Sm	0.02	ICP-MS	100	0.02 (+/- 0)
Eu	50	ICP-MS	Below D/L	N/A
Gd	0.01	ICP-MS	100	0.02 (+/- 0)
Tb	0.01	ICP-MS	Below D/L	N/A
Dy	0.01	ICP-MS	50 - 100	0.02 (+/- 0.01)
Ho	0.01	ICP-MS	Below D/L	N/A
Er	0.01	ICP-MS	100	0.01 (+/- 0)
Yb	0.01	ICP-MS	100	0.01 (+/- 0)
Lu	0.01	ICP-MS	Below D/L	N/A
Hf	0.1	ICP-MS	Below D/L	N/A
Ta	0.1	ICP-MS	Below D/L	N/A
Pb	1	XRF	80 - 100	9 (+/- 1)
Bi	0.1	ICP-MS	Below D/L	N/A
Th	0.1	ICP-MS	Below D/L	N/A
U	0.1	ICP-MS	Below D/L	N/A

2.4 DETRITAL CONTAMINATION

Detrital inputs to vegetation can occur due to wind-borne, non-mineralised and mineralised dust. Non-mineralised aeolian particulates adhering to the surface of vegetation can dilute the biogeochemical expression of mineralisation (Dunn, C. 2003, pers.comm., 28th November). Aeolian sediments are widespread and abundant at all of the study sites, expressing a high potential for dust transport and deposition within vegetation. For instance, the western margins of Racecourse Creek (Tibooburra) are flanked by an extensive aeolian sandsheet,

while the eastern margins of Pine Creek (Broken Hill) are flanked by an aeolian longitudinal dunefield, related to the predominance of westerly winds. Another possible source of detrital contamination is wind blown dust from proximal mine workings (Barrier Pinnacles), and relic gold diggings (Tibooburra) that could potentially elevate the concentrations for mineralisation elements in the analytical results.

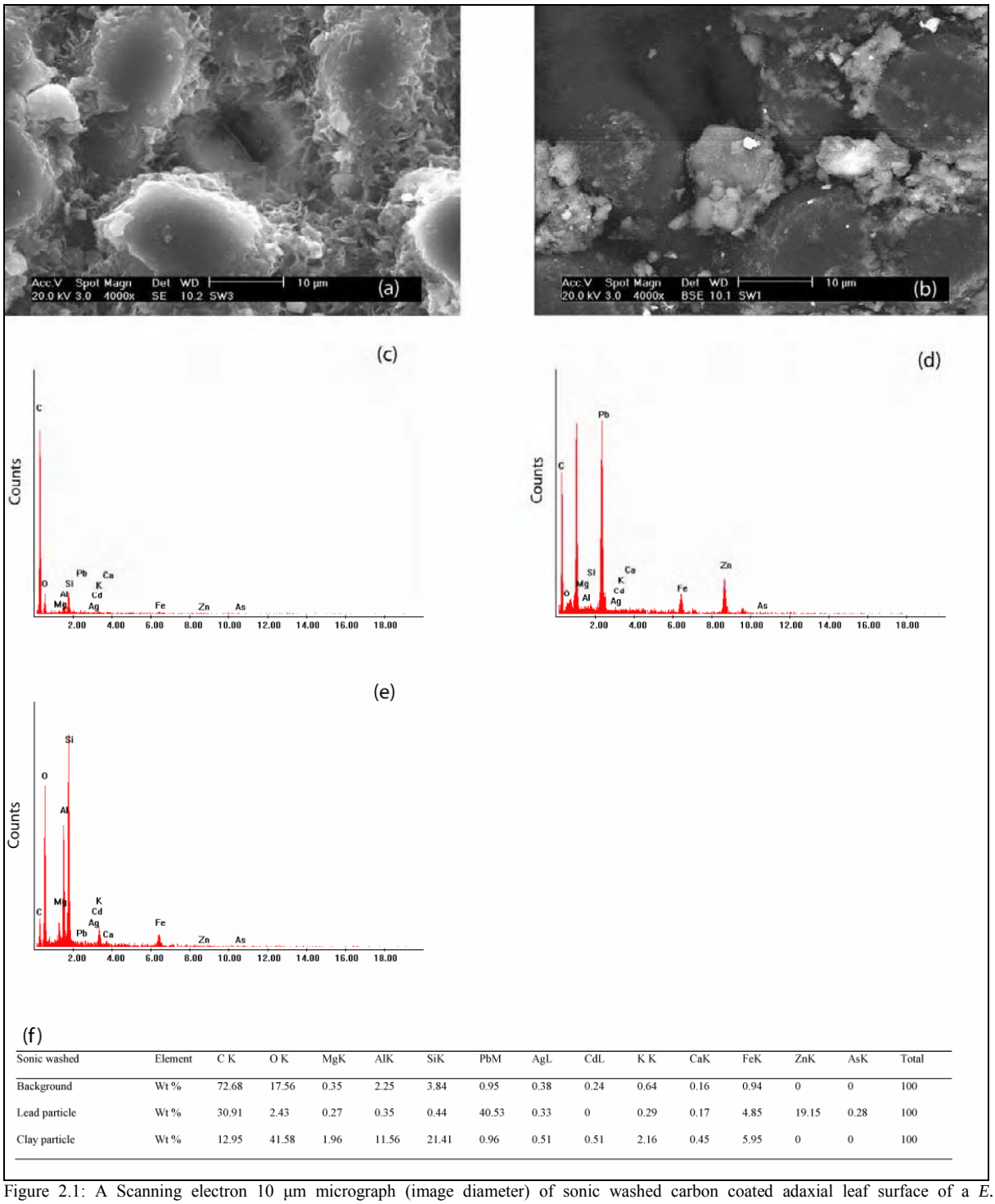
It is therefore essential to ascertain whether the sample is significantly affected by detrital contamination. To determine the significance of detrital contamination on plant biogeochemical results, previous studies (Allen, 1974, Bargagli, 1998 and Brooks, 1998) suggest the approach of using soil contamination chemical markers such as Al, Ti, Sc, Zr, Fe or Si. In order to ascertain the significance of detrital contamination it is important to compare plant tissue concentrations with that of adjacent regolith (stream sediment) concentrations. In this way Al, Ti, Sc, Zr, Fe and In are investigated and compared for the vegetation and stream sediments.

Vegetation samples from the six regional sites (Yunta, Bindarrah, Flying Doctor, Teilta, Williams Creek and Tibooburra) have analytical results below analytical detection limits for Ti (5 ppm – ICP-OES), In (0.01 ppm – ICP-MS) and Zr (20 ppm INAA), suggesting that detrital contamination is insignificant. Correlation plots between vegetation and stream sediments at all six sites were constructed using the Spearman Ranking coefficient. This method was chosen due to its ability to be applied to data that are not normally distributed (Rollinson, 1993). There were few linear elemental relationships between vegetation and stream sediments for the geochemical markers of detrital contamination. Of the suggested elements, poor correlations (<0.50) were obtained between Al, Fe, Ti and In for Bindarrah, Flying Doctor, Williams Creek and Tibooburra, suggesting that detrital contamination in these areas is insignificant (Appendix A). This is not surprising given the characteristics of the *E. camaldulensis* leaves, which are large, smooth, and waxy and therefore hold minimal detrital material. Study sites at Yunta and Teilta, however, may be more significantly affected by detrital contamination. Both of these sites had a strong correlation with the detrital contamination markers, such as Yunta Al – Ti ($r = 0.79$), Teilta Al – Ti ($r = 0.90$) (Appendix A).

E. camaldulensis trees proximal to the Barrier-Pinnacles Mine had elevated concentrations of Pb, Ag, Zn and Cd (Chapter 4). Additional investigations into possible surficial leaf contamination in samples from these trees were undertaken at Adelaide Microscopy. The *E. camaldulensis* with the greatest concentrations of the above elements was selected for investigation. Samples were split, with one sample split sonic washed for one hour, and the other sample split remained unwashed. Both samples were dried according to the described methods (see section on SAMPLE PREPARATION (PRE-DIGESTION)) and carbon coated for examination with a Philips XL30 Field Emission Gun Scanning Electron Microscope equipped with an EDAX DX4 integrated Energy Dispersive X-ray Analyser with mapping capability, HKL Channel 5 Electron Back Scatter Diffraction System (EBSD). Surface secondary electron images (SEM) photomicrographs and backscattered electrons images (BSE) were obtained. The BSE function is dependent on the mean atomic number of the surface atoms, producing images, which provide information about elemental distribution in the sample. An energy dispersive X-ray spectrum (EDS) was collected from selected areas to give possible background concentrations, along with areas of interest identified in the backscatter images.

Scanning electron photomicrographs shown in Figure 2.1 (a) and Figure 2.2 (g) show the general appearances of the adaxial surface of the *E. camaldulensis* leaf (waxy rods). A minor part of the leaf surface area contains detrital particles, but there appears to be little difference between unwashed and sonic washed samples (Figure 2.1 (a) and Figure 2.2 (g)). The detrital particles ranged in size from approximately 6.25 – 12.5 μm. Sulphides and silicates were the two main types of particles. The chemical composition of the particles deposited on both samples (unwashed and sonic washed) is shown by their X-ray spectrum (Figure 2.1 (c – e) and Figure 2.2 (i – k)), and accompanying acquisition data (Figure 2.1 (f) and Figure 2.2 (l)). Figure 2.1 (b) and Figure 2.2 (h) show a cubic Pb-Zn-Fe-rich and plate-like Si-Al-Mg-rich fine particles both approximately 2.5 μm. The Pb-Zn-Fe- rich fine particle is indicative of the Ag-Pb-Zn sulphides currently being recovered from the Barrier Pinnacles Mine. The most likely source for the Pb-Zn-Fe-rich fine particle is the recently excavated costeans (which happened after the main period of plant sampling) and the proximal location of the *E. camaldulensis* to the mine.

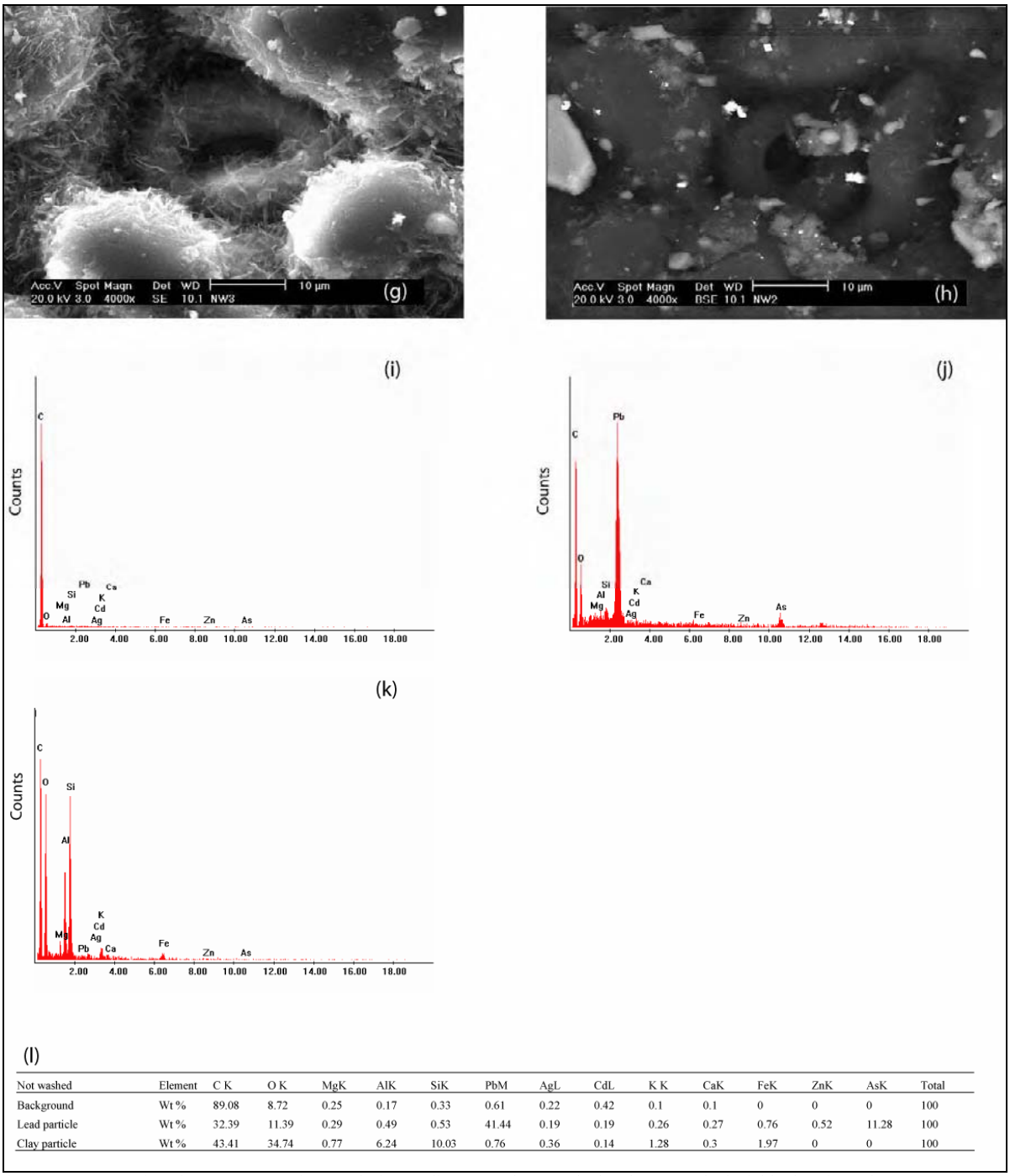
The plate-like Si-Al-Mg-rich fine particles for the most part are possibly derived from the surrounding landscape by alluvial and aeolian dispersion. Stable primary minerals (quartz and heavy accessory minerals) and secondary minerals (Fe and Mn oxides) and weathered transported detrital fragments derived from distal and proximal rocks constitute a large proportion of the regolith materials here. The composition for both samples (Figure 2.1 (c) and Figure 2.2 (i)) was identified as “background material” as it predominately consisted of carbon and oxygen (Tables: f and l within Figure 2.1 and Figure 2.2) with small concentrations of magnesium and aluminium.



(f)

Sonic washed	Element	C K	O K	MgK	AlK	SiK	PbM	AgL	CdL	K K	CaK	FeK	ZnK	AsK	Total
Background	Wt %	72.68	17.56	0.35	2.25	3.84	0.95	0.38	0.24	0.64	0.16	0.94	0	0	100
Lead particle	Wt %	30.91	2.43	0.27	0.35	0.44	40.53	0.33	0	0.29	0.17	4.85	19.15	0.28	100
Clay particle	Wt %	12.95	41.58	1.96	11.56	21.41	0.96	0.51	0.51	2.16	0.45	5.95	0	0	100

Figure 2.1: A Scanning electron 10 µm micrograph (image diameter) of sonic washed carbon coated adaxial leaf surface of a *E. camaldulensis* (a); Scanning electron backscatter 10 µm micrograph (image diameter) of not washed carbon coated adaxial leaf surface of a *E. camaldulensis* (b); EDX – spectrum of background leaf composition (c); EDX – spectrum of a lead-rich fine particle (d); EDX – spectrum of a clay particle (e); EDX – spectrum automatic acquisition data (f).



(l)

Not washed	Element	C K	O K	MgK	AlK	SiK	PbM	AgL	CdL	K K	CaK	FeK	ZnK	AsK	Total
Background	Wt %	89.08	8.72	0.25	0.17	0.33	0.61	0.22	0.42	0.1	0.1	0	0	0	100
Lead particle	Wt %	32.39	11.39	0.29	0.49	0.53	41.44	0.19	0.19	0.26	0.27	0.76	0.52	11.28	100
Clay particle	Wt %	43.41	34.74	0.77	6.24	10.03	0.76	0.36	0.14	1.28	0.3	1.97	0	0	100

Figure 2.2: A Scanning electron 10 µm micrograph (image diameter) of not washed carbon coated adaxial leaf surface of a *E. camaldulensis* (g); Scanning electron backscatter 10 µm micrograph (image diameter) of not washed carbon coated adaxial leaf surface of a *E. camaldulensis* (h); EDX – spectrum of background leaf composition (i); EDX – spectrum of a lead-rich fine particle (j); EDX – spectrum of a clay particle (k); EDX – spectrum automatic acquisition data (l).

2.5 SAMPLE PREPARATION (PRE-DIGESTION)

2.5.1 Sample drying

Sample preparation (pre-digestion) involves a number of stages before chemical analysis. This includes decisions on whether to wash the samples, how to dry or ash the samples, and the subsequent homogenisation and physical breakdown of the sample (grinding/milling). Many of these approaches are governed by the method of digestion and later chemical analysis.

Whether to wash samples or not is a contentious decision for biogeochemical researchers. The main intention of sample washing is to remove surface contaminants, such as detrital particles. There are, however, several potential disadvantages to this, including leaching of biogeochemical components, variable degrees of washing effectiveness, and contamination from further laboratory handling and washing solutions. Table 2.5 shows data from washed and unwashed portions of three different *E. camaldulensis* trees sampled along Pine Creek adjacent to the Pinnacles Ag-Pb-Zn mineralisation, southwest of Broken Hill. In this test the washed samples were sonic washed in de-ionised water for one hour.

Table 2.5: The comparison of unwashed and washed leaves of three *Eucalyptus camaldulensis* samples displaying the effects of sonic washing (one hour) in deionised water on the relative chemical composition of the samples.

Elements (ppm)	**Unwashed**	**Washed**	**Unwashed**	**Washed**	**Unwashed**	**Washed**
	Pine Creek 5b		**Pine Creek 6**		**Pine Creek 6a**	
As	7	8	4	6	6	6
Fe	252	288	270	289	225	286
K	4650	4670	4970	4940	5570	5700
Ag	0.8	1.1	0.5	0.5	1	1.1
Na	2670	2780	1330	1260	2480	2490
Cd	1	1	1	1	4	4
Cu	19	19	14	16	12	15
Pb	539	464	207	266	354	383
Zn	168	148	106	132	215	269

There were no significant decreases in the assay values for washed samples compared to the equivalent unwashed ones. Some elements (e.g. As, Fe, Ag, Pb, Zn) are in greater concentration in the washed samples (Figure 2.3). Possible explanations for this may be that if the elemental concentrations in the leaves are highly variable, then the difference between washed and unwashed samples could be within the range of the natural variation for those elements; or the element is concentrated in a part of the leaf that does not react with sonic washing (i.e. in the veins rather than the leaf tissue or if it is incorporated into the leaf surface wax it may be concentrated due to relative accumulation); or if the detrital components removed by sonic washing did not contain the elements in question and therefore washing increased the elemental concentration by relative accumulation. Similar elevated assays in washed samples were also obtained by Dunn (2003, pers.comm., 28th November) with respect to biogeochemical U assays. The elevated response in the washed samples in that case was attributed to the removal of silicate dust during washing, which prior to washing diluted the expression of U contents within the plant tissue.

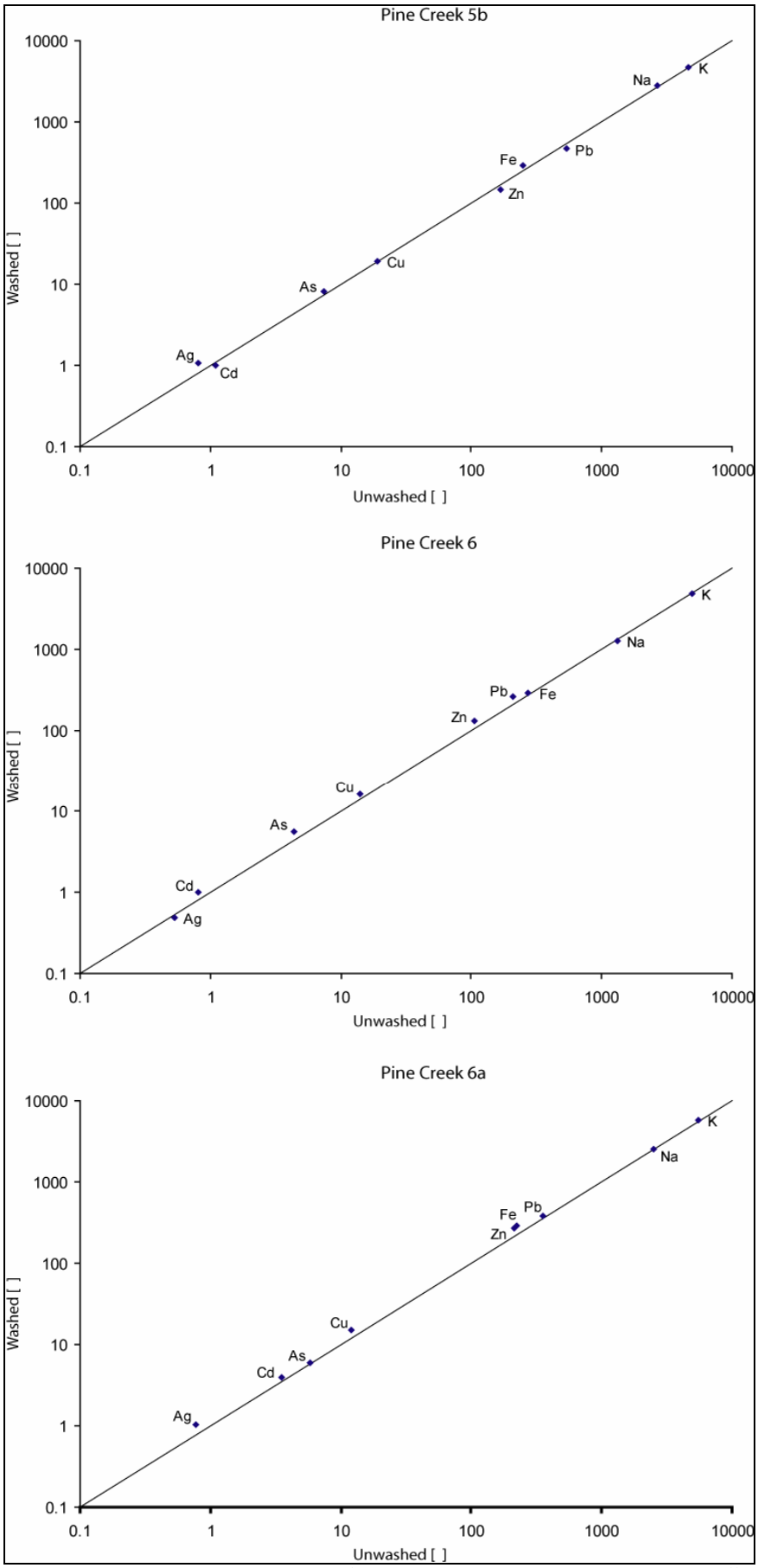


Figure 2.3: Comparison between unwashed and washed leaves of three *E. camaldulensis* samples displaying the effects of sonic washing (one hour) in deionised water on the relative chemical composition of the samples. Black-line denotes a 1:1 ratio.

This study opted not to wash further samples during preparation. The large, smooth, waxy and vertically hanging leaves of the *E. camaldulensis* appear to be a poor repository for detrital material.

The main purpose of sample drying is to ensure that enzyme activity and organic decompositional process are stopped. No ideal temperature or drying time is universally recommended however the three main procedures performed in previous studies when drying vegetation matter include:

- 105 $^{\circ}$ C for 24 hours (Lintern *et al*., 1997);
- 40-50 $^{\circ}$ C for 5 days (Arne *et al.*, 1999); and,
- freeze drying (Bargagli, 1998).

Other recommended drying temperatures and drying times vary from 24 hrs at 100 ^{O}C (Dunn *et al.*, 1995) to 4 hrs at 500 ^{O}C (ashing) (Brooks *et al.*, 1985).

Drying not only makes samples more conducive to long-term storage but it also increases the concentration of many elements to be analysed, at the very least due to the loss of water. A major disadvantage of high temperature drying (ashing) however is that significant losses of moisture are also likely to result in the loss of volatile and in some cases important pathfinder elements, such as Se, Hg and As (Dunn, 1995).

In this study, samples of *E. camaldulensis* were dried in a oven for 48 hrs at 60^{O}C in accordance with Hill (2002).

2.5.2 Homogenisation and fragmentation (grinding)

An important step in the overall analytical procedure is to ensure that the sample submitted for analysis is homogenised and thereby is representative of the original larger sample. There are four main methods used for the grinding of dried biological samples (Allen, 1974). The grinding procedure used in this study is in accordance with those at Becquerel Laboratories, Lucas Heights, New South Wales (Garnett, D. 2003, per. comm., 11th February). The grinder used is a Breville 'coffee-n-spice' grinder CG-2, which has rotating cutting blades. The grinder has a touch activated on/off switch, a transparent plastic lid, a 75 g sample capacity and stainless-steel base & blades with deflection ribs that help to provide an even grind. The full sample is ground. The grinder is then cleaned with compressed air, then ethanol (ethanol 100%, undenatured; C_2H_5OH; density 0.79g/ml) and then further compressed air to reduce cross-sample contamination.

2.6 SAMPLE PREPARATION (DIGESTION) AND ASSAY

In order to obtain a large elemental analytical suite, several techniques were used due to the different strengths, weaknesses and best detection for each technique.

2.6.1 Inductively Coupled Optical Emission Spectrometry (ICP-OES)

A modified aqua regia digest ICP-OES (Genalysis Laboratories, Western Australia) was used to provide biogeochemical assays of the following elements with the following lower

detection limits: Al (20 ppm), Cu (1 ppm), Mg (20 ppm), Mn (1 ppm), Ni (1 ppm), P (20 ppm), S (10 ppm), Ti (5 ppm), V (2 ppm) and Zn (1 ppm).

The procedure for sample digestion is as follows:

1. weigh 0.300 (±0.002) g of homogenised material into a Teflon beaker;
2. add 20 ml of nitric acid (AR grade 70 % m/m) and allow to digest overnight;
3. next morning heat to 105° C until the brown fumes of nitrogen dioxide (NO_2) disappear;
4. allow to cool, and then add 4 ml of perchloric acid (AR grade 70 % m/m) and digest at 105°C until all nitric acid has been driven off;
5. increase the temperature to 180° C and keep heating until about 0.5 ml of perchloric acid or less is left in the beaker;
6. allow to cool, add 1 ml of nitric acid, 10 ml of hydrochloric acid (AR grade, 32 % m/m), and 10 ml of doubly deionised water;
7. allow to cool, and make up to 30 ml with doubly deionised water; and
8. solution is presented directly to ICP-OES for the determination of the selected element suite.

The following stream sediment fractions (<75 μm and 75-300 μm) were analysed by mixed acid digestion followed by ICP-OES (3E), at Amdel Laboratories, Adelaide South Australia. Due to commercial in-confidence reasons, analytical procedures described for analysis at Amdel is a composite of available information released by Amdel and available literature (Tucker, 1988; Potts, 1987).

The following lower detection limits were achieved: Al (10 ppm), Ba (5 ppm), Ca (10 ppm), Cr (2 ppm), Cu (2 ppm), Fe (100 ppm), K (10 ppm), Mg (10 ppm), Mn (5 ppm), Na (10 ppm), Nb (5 ppm), Ni (2 ppm), P (5 ppm), Pb (5 ppm), S (50 ppm), Ti (10 ppm), V (2 ppm), Zn (2 ppm).

The procedure for ICP-OES (3E HF/multi acid digest) digest is as follows:

1. weigh 20 g of homogenised material into vessel;
2. add 15 ml of hydrofluoric acid and 4 ml perchloric acid, allow to digest the main component of sample submitted, the addition of the perchloric acid and fuming (displaces excess hydrofluoric acid);
3. remaining residual is leached with a mixture of 4:1 hydrochloric and nitric acid to take up the precipitated perchlorate salts;
4. solution is diluted to a known volume with distilled water; and,
5. solution is presented to a precalibrated ICP-OES spectrometer for determination of required elements.

A Perkin-Elmer Optima instrument was used to run the analysis.

2.6.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS analysis was conducted on the plant samples at Geoscience Australia (GA) Canberra and Genalysis Laboratories (Perth), Western Australia. With the following lower detection limits:

- ICP-MS: Be (0.1 ppm), Bi (0.01 ppm), Cd (0.1 ppm), Ga (0.1 ppm), In (0.01 ppm), Nb (0.05 ppm), Nd (0.01 ppm), Pb (2 ppm), Sn (0.1 ppm), and Sr (0.05 ppm).

The procedure for vegetation preparation for ICP-MS at Geoscience Australia is as follows:

1. weigh approximately 100 mg of dried homogenised vegetable material into a Savillex Teflon vessel;
2. add 5 ml of distilled HNO_3 and 1 ml of distilled HF;
3. heat for 12 hours, usually overnight, at 120° C on a timed hot plate;
4. very carefully transfer the contents to volumetric flasks; and,
5. make up to 200 ml volume with polished water.

The procedure for vegetation preparation for ICP-MS at Genalysis Laboratories is as outlined for ICP-OES with the solution requiring a further 1:10 dilution of doubly deionised water.

Both Genalysis Laboratories, Western Australia and Geoscience Australia, Canberra use a Perkin Elmer/Sciex Elan 6000 ICP-MS system.

The lower detection limits for aqua regia digest ICP-MS for stream sediment analysis at Amdel Laboratories, Adelaide South Australia, are as follows:

- ICP-MS (3M-mix acid digest, including HF): Ag (0.1 ppm), As (0.5 ppm), Bi (0.1 ppm), Cd (0.1 ppm), Co (0.2 ppm), Cs (0.1 ppm), Ga (0.1 ppm), In (0.05 ppm), Mo (0.1 ppm), Rb (0.1 ppm), Sb (0.5 ppm), Se (0.5 ppm), Sn (0.1 ppm), Sr (0.1 ppm), Te (0.2 ppm), Th (0.02 ppm), Tl (0.1 ppm), U (0.02 ppm), W (0.1 ppm) and Y (0.05 ppm); and ,
- ICP-MS (3R-rare earth elements by mix acid digest, including HF): Dy (0.02 ppm), Er (0.05 ppm), Eu (0.02 ppm), Gd (0.05 ppm), Ho (0.02 ppm), Lu (0.02 ppm), Pr (0.05 ppm), Tb (0.02 ppm), Tm (0.05 ppm), Yb (0.05 ppm), La (0.5 ppm), Ce (0.5 ppm), Nd (0.02 ppm) and Sm (0.02 ppm).

The procedure for stream sediment preparation for aqua regia digest ICP-MS at Amdel Laboratories is as follows:

1. sample is dissolved in a mixture of molten lithium meta- and tetra-borates;
2. resultant glass is leached out in dilute HNO_3.; and,
3. sample is presented to a precalibrated ICP-MS spectrometer for determination of required elements.

A Perkin Elmer/Sciex Elan 6000 quadrupole instrument was used for the analysis.

2.6.3 Atomic Absorption Spectrometry (AA 10)

Atomic Absorption spectrometry was used for the Au analysis in the aqua regia digest from the stream sediments. The lower detection limit for Au is 1 ppb.
The procedure for stream sediment preparation for Atomic Absorption (AA10) at Amdel Laboratories, Adelaide, South Australia is as follows:

1. weigh 50 g of homogenised material into a beaker;
2. aqua-regia digest mixture of (HCl-HNO_3 2:1v/v);
3. sample is heated at a selected temperature; and,
4. Au is attacked by the chlorine gas formed during the decomposition of hydrochloric acid.

A Varian 10 was used for the Atomic Absorption spectrometry analysis of Au.

2.6.4 X-Ray Fluorescence (XRF)

XRF analysis was conducted through Geoscience Australia (GA), Canberra with the following lower detection limits: Al (0.001 %), Ba (8 ppm), Ca (0.002 %), Cl (0.001 %), Cu (0.8 ppm), Fe (0.002 %), K (0.002 %), Mg (0.004 %), Mn (0.001 %), Na (0.004 %), Ni (1.3 ppm), P (0.001 %), Pb (0.5 ppm), Rb (0.3 ppm), S (0.001 %), Si (0.006 %), Sr (0.5 ppm), Zn (0.5 ppm) and Zr (0.4 ppm).

The procedure for vegetation preparation for XRF at Geoscience Australia is as follows:

1. weigh 10 g of milled homogenised vegetation sample;
2. transfer the sample to a 32 mm die and press to 10 ton per square inch in the Spectro press;
3. extract the pellet from the die and stand it on its side on a piece of paper towel. **Do not touch pellet on either face** (Pyke, 2005, pers.comm.). After about an hour the sample number can be written along the pellets side with a fine marker. Sample is left over night to dry and is then transferred to a numbered plastic bag prior to analysis; and,
4. thoroughly clean the die with warm water and dry to minimise cross contamination.

Geoscience Australia (GA) laboratories use a Philips PW2404 4kW sequential spectrometer with a Rh tube.

2.6.5 Instrument Neutron Activitation Analysis (INAA)

INAA analysis was conducted through Becquerel Laboratories, Sydney (Australia) and Becquerel Laboratories, Mississauga Ontario (Canada). INAA allowed for the detection of Au and 33 other trace elements with the following lower detection limits:

- Au (0.1 ppb), Sb (0.02 ppm), As (0.05ppm), Ba (10 ppm), Br (0.2 ppm), Cd (0.5ppm), Ca (0.5 %), Ce (0.2 ppm), Cs (0.2 ppm), Cr (1 ppm), Co (0.5 ppm), Eu (0.1 ppm), Hf (0.2 ppm), Ir (5 ppb), Fe (100 ppm), La (0.2 ppm), Lu (0.02 ppm), Mo (0.1 ppm), Ni (5 ppm), K (0.1 %), Rb (1 ppm), Sm (0.02 ppm), Sc (0.05 ppm), Se (1 ppm), Ag (0.5 ppm), Na (100 ppm), Ta (0.1 ppm), Te (5 ppm), Th (0.1 ppm), W (0.1 ppm), U (0.05 ppm), Yb (0.5 ppm), Zn (10 ppm), and Zr (20 ppm).

The procedure for vegetation preparation for INAA is as follows:

1. weigh 10 g of milled homogenised vegetation sample and flux monitor to each sample;
2. transfer the sample to a 32 mm die and press to 20 ton per square inch in a SPEX X-PRESS;
3. samples are then shrink wrapped to prevent contamination;
4. samples are stacked into one-foot long bundles for irradiation; and,
5. each bundle randomly contains selected duplicates at the base of the bundle and standards inserted at random positions.

Bundles are irradiated for 20 minutes in the core of a nuclear reactor at a flux of 8×10^{12} $n/cm^2/s^{-1}$, the bundles are rotated during irradiation so that there is no horizontal flux variation. Vertical flux variation is monitored with the individual flux monitors. After a decay period of six days, the irradiated samples are loaded onto the counting system. High resolution, coaxial germanium detectors are used. The counting time is twenty to thirty

minutes per sample. After an additional decay period of at least ten days, the samples are recounted for one hour.

2.6.6 Microscopy

Prior to laser ablation ICP-MS micro-analysis, samples were sectioned across the leaf using a surgical scalpel and mounted onto microscope cover slips. Identification of the internal structures of the *E. camaldulensis* (Figure 2.4) leaf was identified using a Nikon Eclipse TE300 Inverted Microscope, and photographed.

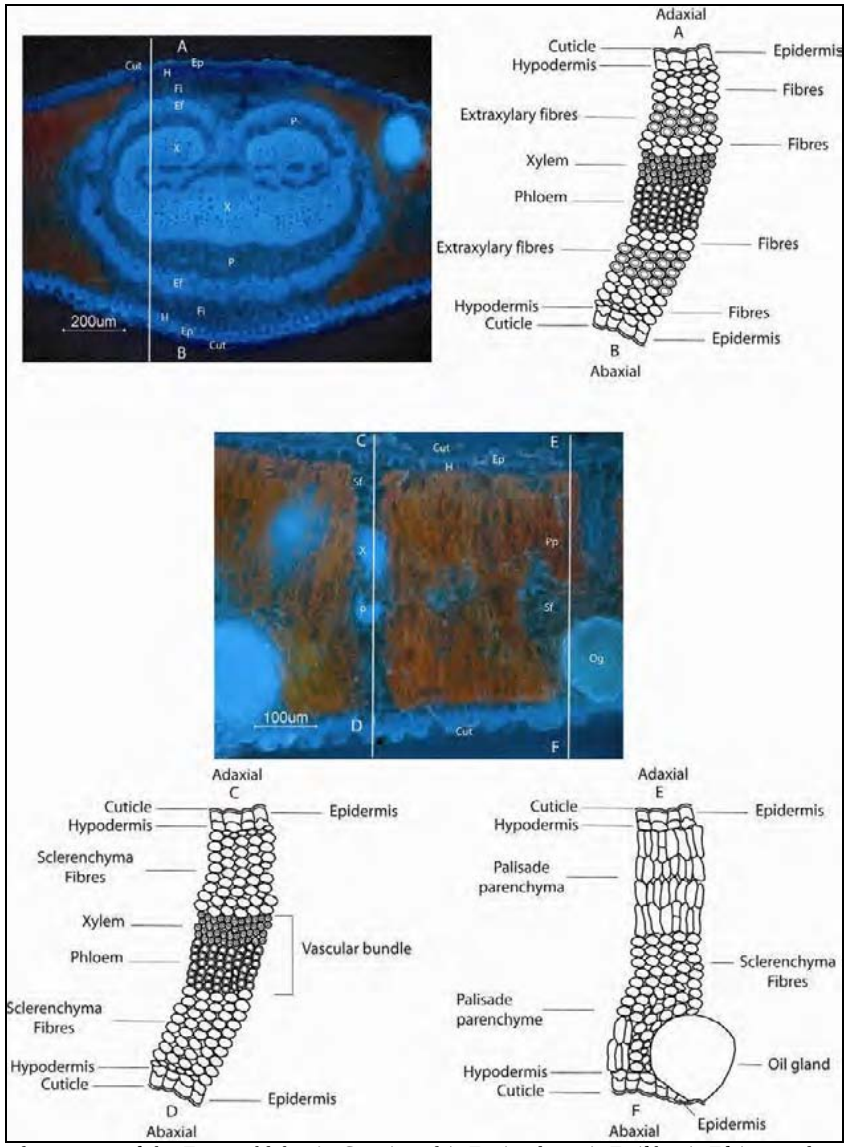


Figure 2.4: The Internal structures of the *E. camaldulensis*. Cut- (cuticle), Ep (epidermis), Fi (fibres), Ef (extraxylary fibres), X (xylem), P (phloem), Sf (sclerenchyma fibres), Pp (palisade parenchyma) and Og (oil gland). Sections A-B, C-D and E-F indicate where the cross section and accompanying illustration are derived from.

2.6.7 Laser ablation ICP-MS

The *E. camaldulensis* trees proximal to the Barriers-Pinnacles Mine that had elevated concentrations of Pb, Ag, Zn and Cd had additional investigations into the compartmentation/partitioning of metal ions within the *E. camaldulensis* leaf. Adelaide Microscopy's Agilent 7500cs ICP MS was used for the analysis of a *E. camaldulensis* leaf cross section, in conjunction with a high performance laser ablation (New Wave Nd Yag 213 UV), for the analysis of the following relatively stable isotopes:

- Al^{27}, Si^{28}, S^{32}, S^{34}, Ca^{43}, Ca^{44}, Fe^{54}, Fe^{56}, Fe^{57}, Mn^{55}, Co^{59}, Ni^{60}, Cu^{63}, Cu^{65}, Zn^{64}, Ge^{74}, As^{75}, Sr^{88}, Ag107, Cd^{110}, Cd^{114}, In^{115}, Sb^{121}, Ce^{140}, Au^{197}, Pb^{206}, Pb^{207} and Pb^{208}.

The sample was collected the day before the analysis to ensure that there was minimal internal leakage of metal ions from one cell structure to the other, and further ensuring that all results are both qualitative and quantitative. Figure 2.5, shows where the leaf cross-section was taken. This section was chosen as all cells here should be fully developed.

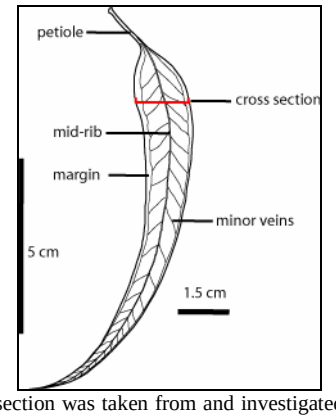


Figure 2.5: Shows the location of were the cross section was taken from and investigated, and anatomical features of the *E. camaldulensis* leaf.

Samples were sectioned across the leaf using a surgical scalpel and mounted onto microscope cover slips using paraffin oil to support the leaf. It is essential to produce a flat, even cross-section across the entire leaf to ensure that the ablation conditions are uniform. The microscope cover slips were introduced into the Ablation cell of the New Wave UP213 nm Nd-YAG laser system and purged with UHP Helium. The ablated material from the helium gas stream is blended with argon carrier gas, before being introduced to the Agilent 75oocs ICPMS system. Prior to each ablation, the laser is fired for 30 s with gas with the shutter closed to ensure that the crystal and beam are stable; this was followed by 50s of measurement during *E. camaldulensis* ablation.

Data acquisition parameters include:

- spot size- 25 µm;
- repetition rate- 5Hz; and,
- energy density on sample- 3J/cm^2.

Once the sample was introduced into the Ablation cell, 10 points of ablation were taken across specific cell structures (Figure 2.6).

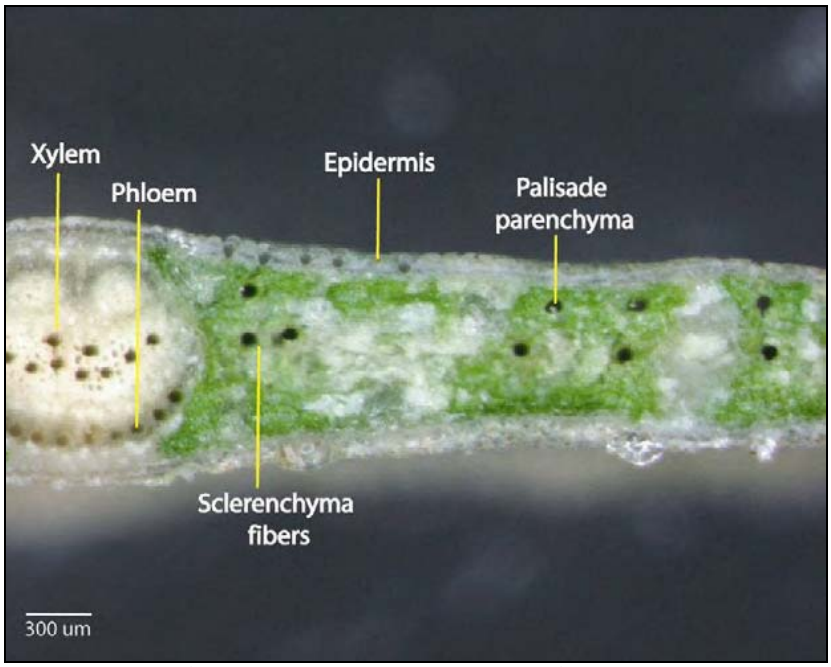


Figure 2.6: Image shows the location of laser ablation points across the identified internal structures, epidermis, xylem, phloem, sclerenchyma fibres and palisade parenchyma cells.

2.7 STATISTICAL ANALYSES

Stastical analysis allows for large volumes of data to be compressed into manageable representative values, enabling valid conclusions to be established. To ensure that results and their interpretations are meaningful and comparable, the following stastical methods have been applied to the biogeochemical surveys at the Pinnacles (Pine Creek) and Tibooburra (Racecourse Creek).

Student's T-test

The Student's T-test is a statistical method that assesses whether the means of two groups are statistically similar or different from each other. In addition, the following null hypothesis was adopted **"There are no significant differences between the means and that both groups are derived from the same population"**, at a significance level of 0.05. The results revealed that the null hypothesis was rejected and both groups are not derived from the same population. Therefore, it is suggested that there are two groups (populations) at both the Pinnacles and Tibooburra. Theses groups appear to correspond to:

- Tibooburra (Racecourse Creek): *E. camaldulensis* adjacent to the granodiorite and those flanking the Metasediment (Chapter 4), and;
- Pinnacles (Pine Creek): *E. camaldulensis* neighbouring the Barrier Pinnacles mineralisation and those thought to be associated with the surrounding host rocks (Chapter 4).

In order to define the two popoulations the data were subjected to futher statistical analysis through the application of principal components analysis.

Principal component analysis

Principal component analysis is a statistical approach that can be used to analyse inter-relationships for a large number of variables and to explain these variables in terms of their common underlying dimensions (factors). The statistical approach involves finding a way to

condense the information contained in a number of original variables into a smaller set of dimensions (factors) with a minimum loss of information (Hair, *et al*., 1992).

In conjunction with multivariate analysis, univariate analysis was performed on each individual element from both the Pinnacles (Pine Creek) and Tibooburra (Racecourse Creek).

Univariate statistics

Univariate statistics involve the investigation of each individual variable (e.g. element). This can help to futher identify the type of distribution of the data, and therefore the presence of one or multiple populations and the chance of outliers. This was achieved through the construction of box and whisker (Tukey) plots. In these plots: the box represents approximately 50 % of the data and is enclosed between the upper and lower hinge: and, the median is represented by a horizontal line, which from its position depicts the skewness or symmetry of the data (Bounessah & Atkin, 2003). The "whiskers" indicate the peripheral data, each representing 25 % of the population. Data that extends beyond the whiskers are defined as outliers and are represented by a blue triangle (in this study), while a red circle represents very extreme outliers (Figure 2.7). A two-sigma error plus standard deviation was applied to define the 95% confidence interval for the concentration range for all elements. In addition, histograms and cumulative frequency plots are included on the biogeochemical maps as they both provide another form of visual representation of the data. All statistical calculation was performed through the use of the software Datadesk®.

Element analytical results that reported less than 20 % of the data below analytical detection limits were replaced with half the lower detection limit value, for the generation of a box and whisker plot (Figure 2.6). Analytical results for an element that had more that 25 % of the population below analytical detection limit were removed and the variable removed from further analyses. For the production of histograms, cumulative fraction plots and summary statistics, element analytical results that reported less than 20 % of the data below analytical detection limits were removed (leaving cell blank). The removal of the below analytical detection limit values in the generation of histograms, cumulative fraction plots and summary statistics reduces the potential exaggeration and therefore misleading conclusions. These assigned values, however, are a statistical artefact and need to be recognised, especially when interpreting values approaching or below the lower analytical detection limits.

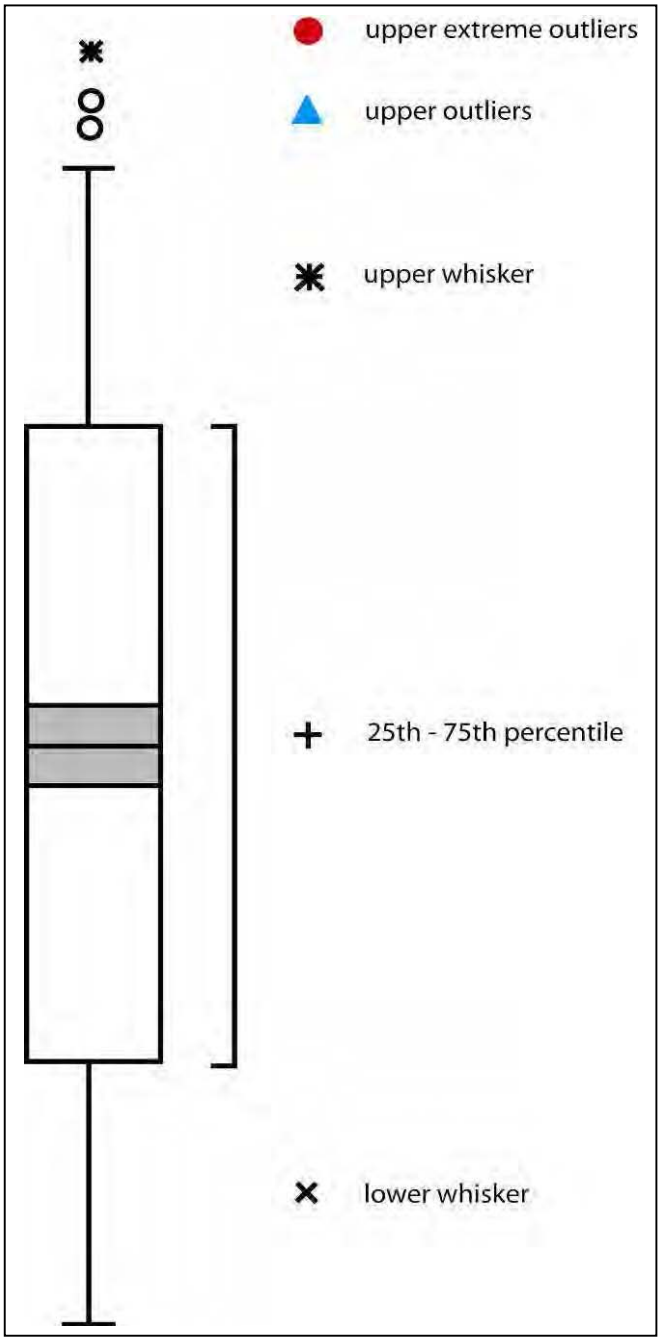


Figure 2.7: Illustration of a Boxplot (or box-an-whisker plots) which is used for statistical depiction of *E. camaldulensis* data. Shapes are assigned to represent data from certain percentiles as marked.

2.8 OVERVIEW OF METHOD SYSTEM (FLOW CHART)

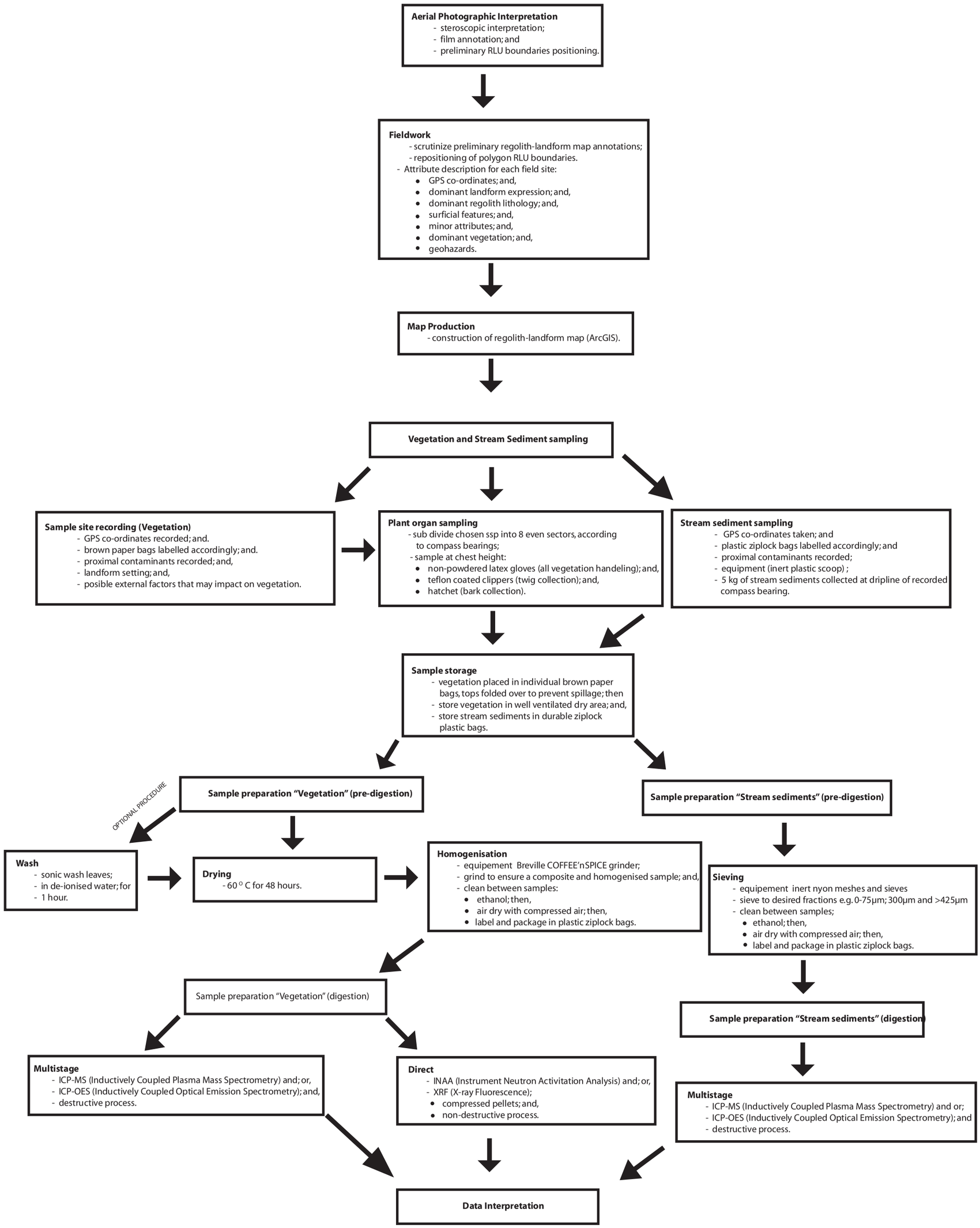


Figure 2.8: A flow diagram of procedures from photo interpretation to data interpretation used throughout this thesis.