# BRANCHING OUT INTO BIOGEOCHEMICAL SURVEYS: A GUIDE TO VEGETATION SAMPLING

L.J. Hill

CRC LEME, Geology Department, Australian National University, Canberra, ACT 0200

Biogeochemical surveys can provide a valuable insight into the dispersion pathways between regolith and vegetation within the landscape. Geochemical sampling surveys may involve the sampling of vegetative material, regolith and bedrock with the sampling process dependent on what the results are to be used for, and constraints on time and resources. This paper provides a guide to sampling vegetation and discusses some of the issues associated with it including contamination and developing a survey design. A robust and relatively easy method of vegetation sampling has also been included.

## SURVEY DESIGN

Designing a sampling program will depend on the aims of the survey and the abundance of selected species. Sampling may be conducted along transects, within a grid, where target species are present (depending on vegetation density) or as a stratified random survey. As a guide, areas liable to contamination should be noted or avoided. Potential contaminants may include metallic objects, buildings, fences, power lines and roadways. Prior to sampling, a detailed geobotanical survey should be conducted. This will include recording vegetation cover, regolith material, landforms, geology and climatic conditions. Depending on the area to be sampled and the complexity of the surface expression, regolith-landform mapping along with detailed vegetation surveys may help to select suitable sampling media and sampling sites. Detailed vegetation surveys may include identifying taxon and recording its cover within a quadrat. As a guide, cover abundance can easily be recorded using the modified Braun-Blanquet scale (Table 1) where an abundance rating is allocated according to a subjective estimation of the percentage cover in the quadrat. The size of quadrats will depend on vegetation density (Cain 1938).

**Table 1**: Modified Braun-Banquet abundance ratings for geobotanical surveys, from Causton (1988).

| Abundance | Braun-Blanquet rating |
|---|---|
| <1% | + |
| 1 – 5% | 1 |
| 5 – 25% | 2 |
| 25 – 50% | 3 |
| 50 – 75% | 4 |
| 75 – 100% | 5 |

## WHAT TO SAMPLE

Selection of plants should be limited to species that are easy to identify, dominant and widespread. Plant uptake can vary according to a number of internal and external factors and so selected plant individuals must be of similar size, age and health so that results are comparable. As every species has different chemical compositions, nutrient requirements and tolerances, comparisons should only be made at the species level unless adequate knowledge of the plant's uptake strategies is known. In addition, each plant organ has a different capacity to store metals and, therefore, comparing the same plant tissue may be the only way to draw valid conclusions. Many researchers have found that above-ground vegetative material provides results that adequately reflect the elemental levels of the entire plant. Moreover, aerial tissues have the advantage of being easy to sample and less handling is required to tackle soil contamination issues. However, certain plants will store trace elements in root material that may be used effectively as an indicator of metalliferous ground.

Regardless of which plant tissues are targeted for sampling, care must be taken to ensure that selected plant organs have no obvious deformities (including presence of faunal products) or desiccation. Moreover, most nutrient concentrations are highest in younger tissues (Allen 1989, Ernst 1995) and so samples for the survey should be restricted to those of similar age and size. Some researchers have separated petioles and blades before analysis (Allen 1989), however, to keep sampling procedures simple bulk leaf samples may be taken. Certainly problems arise when sampling leaves; nutrient levels are reported to vary between sun and shade leaves and the crown position (Allen 1989, Salisbury & Ross 1992). Therefore, it is necessary to sample around the circumference of the plant to gain an estimate of the entire plant chemistry.

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**HOW TO SAMPLE: A SUGGESTED METHOD**

When sampling tree species, samples should be taken at a similar height (chest height is perhaps the easiest) and, along with shrubs, sampled at various points around the circumference of the plant to ensure a representative sample for the entire individual. The amount of sample taken needs to be enough for chemical analyses and also to be representative of the individual's chemistry. The optimum sample size is still debated between researchers but as a guide should be no less than 20 g.

The most important issue for sampling procedures is one of contamination. Plant material is especially open to contamination from a variety of sources and particular care is needed when sampling. Non-powdered latex gloves should be worn at all times when sampling and all jewellery (especially rings) must be removed during the sampling program. Teflon-coated pruning clippers are best used for sampling but should be cleaned regularly with distilled water. Where necessary, a clear or white plastic paint scraper can be used to help remove bark from tree trunks (Dunn *et al*. 1995). It is recommended that, if sampling aerial vegetation, samples be collected as whole branches. Twigs and leaves can be separated with greater ease after drying.

Once collected samples need to be stored. Plant material is likely to sweat and decompose if stored for a reasonably long period of time and this may cause some change in mineral composition. Storage of fresh material in polyethene bags in warm conditions for long periods of time has been known to encourage enzymic reactions which may lead to the breakdown of organic material (Allen 1989) and also volatilisation due to microbial activity. Therefore, it is suggested that samples be stored in brown paper bags. This allows the sample to air and keeps the sample out of direct light, avoiding mould development. Bags also need to be sealed. There are a number of different methods that may be used to seal the bags—folding the top of the bags twice for example. If needed, folds may be secured with staples although extra care needs to be taken to ensure that staples do not come into contact with sample material.

**WHEN TO SAMPLE**

Plants have different nutrient requirements depending on physiological activity. Therefore, their chemical composition will vary throughout the year and from year to year. This affects all living tissue within the plant (excludes bark and older tissues such as wood) and needs to be considered when sampling. For biogeochemical studies in the northern hemisphere sampling in springtime, when plants are most physiologically active, is often recommended (Allen 1989, MacNaeidhe 1995). However, in semi-arid and arid regions of Australia there is no distinct growing season. An optimum season for sampling has yet to be determined for these regions but care should be taken to ensure that sampling programs are conducted in as short a time as possible (over no more than 2-3 weeks depending on conditions and plant material). Some researchers have reported slight diurnal variations in elemental concentrations and recommend sampling during the mid-day period (Allen 1989). This is of course quite an uncomfortable proposition in semi-arid and arid environments. Instead it is important to ensure that the weather conditions are relatively constant throughout the entire time of sampling. Variations due to changes in weather have been noted in some studies (Markert 1995, Brooks 1998).

**PRE-DIGESTION PROCEDURE AND STORAGE**

The pre-digestion procedure may involve the washing, drying and grinding of samples depending on the method of digestion and chemical analysis. Washing of samples for biogeochemical analyses has been used to reduce the effects of locally- and distally-sourced soil contamination on vegetative materials. However, there are no defined rules for cleaning vegetative material and a great variety of cleaning methods have been reported in the literature. Washing with water (tap, deionised, distilled or ultrasonic washing), detergents and diluted acids are the most commonly used methods of cleaning. The efficiency of washing and its effect on plant tissues is difficult to assess and is likely to vary between plant species and tissues (Bargagli 1998, Markert 1995). The effect of washing is also effected by particle-size (i.e. submicron particles are difficult to wash off and may contribute significantly to trace metals levels, while larger particles (> 1 μm) are easily removed). Moreover aerosols from washing water may be absorbed or embedded in waxy outer layers, thus making washing ineffective. Washing may not be applied to some vegetative materials as it causes significant elemental loss from tissues (Bargagli 1998, Brooks 1998, MacNaeidhe 1995, Markert 1995). Clearly this is an area of sample preparation that requires more consideration, particularly its effect on Australian plant species.

Vegetation samples need to be dried to discourage the decomposition of material. Air-dried ground plant material can be stored for long periods of time at room temperature in well-ventilated conditions (Allen 1989). However, the effect of drying on the chemical composition of material needs to be considered. Drying the samples prior to digestion will lead to a significant loss of moisture that will almost certainly be

accompanied by a slight loss of volatile constituents by chemical changes within the sample (Allen 1989). Although this is probably not significant for most species, it is suggested that samples not be dried for more than 48 hours. High temperature drying (above 80°C) for long periods is to be avoided because it causes significant loses in total weight, in volatiles or in charring. Elements that are readily oxidised, such as sulphur, boron and selenium, may be more susceptible to loss. Conversely, temperatures below 40°C won't halt metabolic activity and mould development that can alter elemental levels particularly if the samples are initially damp (Allen 1989, Bargagli 1998, MacNaeidhe 1995). It is suggested here that samples be dried in an oven for 48 hours at 60°C.

When dry, vegetation samples can be more easily separated into leaves and twigs and then, if a wet ashing digestion method is to be applied, ground to a fine (5 mm–63 μm) powder. The risk of contamination during grinding can be quite high, therefore, selecting the right pulveriser is crucial to the success of the survey. The chemical composition of the mill must taken into account. If a broad suite of trace elements is to be analysed then a zirconia mill may prove to be one of the best options for grinding. A Rocklabs™ Nilcra Poly-Stabilised Zirconia (PSZ) 100 g ring mill has been used successfully for biogeochemical surveys (Jones 1999, Senior 2000, Debenham 2000, Dann 2001, Hill *in prep*., Thomas *et al*. 2002) for rapid, fine grinding of material, particularly for moderately hard and brittle material such as twigs and bark. The PSZ mill represents a good trade-off between wear resistance and contamination, as it contains principally Zr, Mg and Hf but is quite wear-resistant. The optimum relation between particle size and sample weight is still debated between researchers but most agree that a particle size of 0.5 mm or less is acceptable (Allen 1989). Sample homogenisation is important to ensure that the sample used in analysis has the same mean chemistry as the sample collected in the field (Markert 1995). As such, care should be taken to ensure that the entire sample, including the final residue, is obtained from the mill. Samples should then be stored in well-sealed containers in the absence of light to reduce the intake of water vapour and avoid fungal infection.

## ROOTING OUT POTENTIAL CONTAMINATION PROBLEMS

There is a broad range of contamination sources that may effect biogeochemical surveys. Some of those sources have already been mentioned: field equipment, skin and jewellery. Vegetative material may also be contaminated from the substrate either from aeolian particles or soil splash. Soil particles can become lodged in plant tissue and can be extremely difficult to remove. Interestingly, Allen (1989) notes that if a sample is contaminated by soil, potassium from plant tissues will readily bind to soil minerals, decreasing potassium levels in the vegetative sample. Identification of the presence of soil contamination (using chemical markers such as Ti) and its possible source can help deduce the effects of contamination on biogeochemical results. Other sources of contamination may include: animal droppings; films of insect products; wind blown fertilisers; smelting practices; metal contamination from nearby structures; and corroded sampling tools. Many of these contaminants can be avoided through careful sampling procedures and careful selection of sampling sites.

## CONCLUSION

To obtain meaningful and comparable results it is important that sampling procedures and analyses are accurate, precise and representative. The sampling method described in this paper is by no means the only possible method of sampling vegetation and may simply be used as a guide to develop sampling procedures. It is important that sampling programs incorporate some attempt to constrain environmental factors such as regolith cover and climatic conditions, and that the sampling process is consistent.

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