BIOGEOCHEMICAL STUDIES ON SOME NICKEL ACCUMULATING PLANTS FROM NEW ZEALAND AND NEW CALEDONIAN SERPENTINE AREAS.

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ABSTRACT

Serpentine areas in New Zealand and New Caledonia are described.

A study was made of soil factors controlling the distribution of five species from a serpentine flora in the Dun Mountain area, South Island, New Zealand.

Samples of soil were taken from sites of each of the species, and each sample was analysed for calcium, chromium, cobalt, copper, magnesium, manganese, nickel, potassium and zinc. On the basis of the species growing on them, the soil samples were divided into five groups: group 1, *Pimelea suteri*; group 2, *Myosotis monroi*; group 3, *Hebe odora*; group 4, *Cassinia vauvilliersii*; group 5, *Leptospermum scoparium*.

Discriminant analysis was used to characterise each group of soils on the basis of chemical composition. The results showed that the two endemic plants (*P. suteri* and *M. monroi*) were much more commonly found in localities of highest magnesium concentration. These two species were strongly differentiated by the potassium and copper levels in their soils. No strong elemental discrimination was found among the non-endemic species.

Correlation coefficients were calculated for the relationships between pairs of elements and highly-significant correlations ($P \leq 0.001$) are reported.

A nickel accumulating species from New Caledonia, *Homalium kanaliense* is compared with the New Zealand nickel accumulator, *Pimelea suteri*.

The very high accumulation of nickel in the New Caledonian species, presents interesting questions in plant physiology. Purification of nickel complexes from an aqueous extract of *B. kanaliense* leaves was achieved and preliminary identification methods employed. None of the nickel was associated with amino acids and the present evidence suggested possible complexing of the nickel to simple carboxyllic sugars.

ACKNOWLEDGEMENTS

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GENERAL
INTRODUCTION

Serpentine and other ultramafic soils through out the World are well known for their unique flora. These soils are easily differentiated from the surrounding lithology, with the 'boundary' lines clearly marked by the abrupt changes in vegetation type. Serpentine regions are typified by extensive areas of sparsely populated stunted vegetation with several species restricted exclusively to these areas with 'disjunctive distributions and boreal affinities' (Whittaker, 1954).

The general infertility of serpentine soils has been known for a long time. The cause of this infertility has been considerably disputed and is still largely unresolved, although it must be kept in mind that reasons applying to one serpentine area may not be applicable to others. However, from time to time, various hypotheses have been advanced to account for the characteristic flora of serpentine soils. They fall broadly into three categories.

Firstly, these soils are generally low in the plant nutrients; calcium, nitrogen, potassium, phosphorous, manganese and molybdenum. Secondly, the unfavourable calcium/magnesium ratio is regarded by many as the dominating factor. The magnesium content of many serpentine soils is often several times the calcium level.

Thirdly, the heavy metal content often approaches and exceeds toxicity levels, particularly those of nickel, chromium and cobolt.

Lipman (1926) has pointed out the unsatisfactory nature of the soil microflora as a possible cause of serpentine infertility.

In a review of some of the literature on this subject it can be seen that there are exponents of

each of these hypotheses, with evidence obtained from work done on serpentine areas throughout the World.

At the beginning of the century, Loew and May (1901) showed that soils having a low calcium/magnesia ratio were much more infertile than high calcium soils. However, Gile (1913) considered that the other soil components were just as much of importance, if not more, as the calcium/magnesium ratio, and that in dilute soil solutions the influence of this ratio was even smaller.

From experiments carried out on the soils of the Great Dyke, Rhodesia, Blackshaw (1921) found that serpentine-derived soils were unproductive whilst soils derived from norite were made to be quite fertile. The serpentine-derived soils were high in magnesium and low in phosphorous and potassium, but large fertilizer applications did not achieve good results. Soame and Saunder (1959) substantiated these findings. Working again on Southern Rhodesian serpentine soils, the deficiency of nitrogen, phosphorous and potassium as the dominant cause of infertility was ruled out, in these areas anyway, because of the lack of response to heavy applications of fertilizers. They also discussed the effect of nickel and chromium toxicity, and possible interactions between chromium, phosphorous and calcium in the soil-plant system. Contradictory to this Gordon and Lipman (1926) concluded that serpentine infertility was caused by the low level of nitrogen, phosphorous and the alkaline reaction, and was not due to the low calcium/magnesium ratio.

The first workers to point out the role of high levels of heavy metals, were Robinson et al (1935), and they stated that this was the dominant cause of infertility. From a detailed physical analysis they

also concluded that serpentine soils possess no physical characteristic which would render them particularly unfavourable for plant growth, although the clay content of some subsoils were high enough to impede drainage (This was noted in the Dun Mountain ultrabasic area). Birrell and Wright (1945) working in a New Caledonian serpentine area came to similar conclusions. They stated that nickel and chromium were responsible for the absence of the common tropical species, and 'species that have established themselves on the serpentine have developed sufficient tolerance to these elements to allow of a low, shrubby growth'.

Mitchell (1945) suggested that nickel was the limiting factor, and he indicated that the amount of nickel extracted by 2.5% acetic acid was the most representative of the nickel status. He put forward the value of 11 ppm soluble nickel which when exceeded would result in toxicity.

It has been found that a number of the Californian serpentine soils are deficient in available molybdenum (Walker 1948), but although Walker (1954) concluded that the molybdenum deficiency may contribute to the infertility of some serpentine soils, it is probably not the dominant factor. The same author suggested from his experimental evidence that the low calcium level is the basic cause of the peculiarity of serpentine soils, with the other factors of secondary importance. However low levels of plant nutrients, alkalinity, low available molybdenum, toxicity of nickel or chromium and the level of calcium and magnesium in the soil all have effect under certain circumstances. In some cases unfavourable physical aspects may also effect serpentine plant distributions.

Hunter and Vergnano (1952) reported that the infertility of serpentine soils at Whitecairns in Aberdeenshire was due to toxic levels of nickel. They found a low level of potassium, phosphorous and sometimes manganese, moderate levels of calcium and high levels of magnesium. The acetic acid-soluble nickel was very high. From experimentation with plant cultures the authors verified that plant growth decreased and necrotic symptoms increased when the calcium magnesium, nitrogen and potassium concentrations were low, and the nickel uptake was proportional to the acetic acid soluble content in the soil. They also compared chlorosis and necrosis due to nickel and cobalt, and while they were similar, they were not identical. Induced nickel symptoms were similar to those of plants with comparable nickel levels from serpentine areas. Cobalt symptoms of sand-cultured plants and necrosis of serpentine-grown plants were not comparable.

Hunter and Vergnano (1953) examined the activity of large amounts of heavy metals in producing chlorosis and other symptoms and found the order to be Ni > Cu > Co > Cr > Zn > Mo > Mn which compares well with the order of stability of metal complexes. Toxic effects of nickel, copper, zinc and manganese were associated with high concentrations of the elements in the leaf tissue, but this was not always so with chromium and aluminium. They suggested one factor in nickel toxicity may have been the inhibition of one or more of the functions of copper, as copper reduces the leaf necrosis produced by nickel, but not the nickel content of the leaf tissue.

Rune (1953) supported the hypothesis of Robinson et al (1935) that the high content of nickel and chromium was the general cause of infertility

in serpentine soils but emphasized that the effect of the elements depended on soil factors such as low nutrient content, high magnesium, pH, mechanical composition etc. Other factors such as low competition, microclimate, topography, physical properties are important in determining the special characteristics of the serpentine flora. These may have varying effects in different areas.

Kruckeberg (1954) supported Walker's (1954) results in showing that tolerance of low calcium levels is a principal adaptation required by serpentine growing plants. He showed that serpentine plants adapted to low calcium, could grow equally well, if not better, on non-serpentine soils but intolerance to competition may have restricted them exclusively to serpentine areas. These results invalidated the ideas of Novak (1928) and Lammermayr (1927) who thought that serpentine plants were restricted to serpentine areas because of some essential requirement obtained only from those soils.

Whittaker (1954) described in detail a serpentine vegetation of the Sishiyou Mountains of South Western Oregon. He suggested three approaches to the problems of serpentines: the edaphic, the soil itself in relation to plant ecology; the autecological, which deals with the response of plant species to serpentine and non serpentine area; and the synecological, which considers the unusual character of serpentine vegetation. Comparing a diorite area with a serpentine vegetation he found that there were very few species that were found on both, and that many serpentine species have probably become serpentine endemics by a process of 'biotype depletion', through losing out in competition with other plants in non-serpentine populations.

Crooke et al (1954) working with sand and water cultures concluded that chlorosis depends on the nickel/iron ratio. The addition of iron lessened the toxicity of nickel and necrosis specifically caused by nickel was correlated with the nickel level in the leaves. This content was significantly reduced by a high concentration of iron in the nutrient solution. This confirms the view of Hunter and Vergnano (1953). Following a similar line Knight and Crooke (1956) studied the interaction between nickel and calcium and results showed how nickel may affect processes within the plant. It may be possible that organic acid metabolism may be affected in nickel toxic plants and that the state of solubility of copper in tissues may be altered by increased production of oxalic acid. They also showed that roots are damaged by nickel and the absorption and translocation of macronutrients is affected.

Soane and Saunder (1959) studing chromium and nickel toxicity in maize and tobacco found that the uptake of chromium in leaves was very slight, although severe root damage and accumulation of chromium in roots occurred. They hypothesized possible chromium, phosphorus and calcium interactions in the soil-plant system and because of this, chromium toxicity is less clearly evident than nickel toxicity. Also chromium is less readily transported to leaves by plants than is nickel.

In a comprehensive spectrographic survey Lounamaa (1956) compared fifteen trace elements in plants growing on silicic, calcareous and ultramafic rocks of Finland. The highest values for chromium, nickel and cobalt were obtained from plants growing on outcrops of ultramafic rocks. For ultrabasic

soils he reported the following means (in p.p.m.); chromium 4000 ± 310, Nickel 1200 ± 190, Cobalt 140 ± 15, copper 22 ± 5.4, Manganese 1200 ± 150. Nickel was seldom observed to be below 1000 p.p.m. He also concluded that nickel and chromium were responsible for the peculiarities of ultrabasic flora.

Spence (1957) working with serpentine soils of the Island of Unst concluded that nickel is the cause of infertility only when associated with other factors such as soil instability and exposure. That is, the sparse colonization of the debris is due not entirely to a 'serpentine effect'. He noted that rapid weathering was associated with a high acetic acid-soluble nickel content.

Krause (1958), Paribok and Alexeyeva-Popova (1966), and Sarosick (1964) suggested that the survival of plants on serpentine soils was dependant on their ability to adapt to all of the factors operating in the serpentine ecosystem and not on one or several of these factors. These factors may operate in varying degrees and combinations depending on the local conditions.

Studing the toxicity and movement of heavy metals in Portuguese serpentine soils Menezes de Sequeira (1968) found that pH, organic matter, copper, nitrogen, potassium and other such factors have a profound effect on the degree of nickel toxicity. Accelerated soil erosion was a consequence of the scanty vegetation cover and detailed reconsideration of the weathering processes may be needed. The author concluded by agreeing with Krause's statement "we cannot mention a unique and omnipotent factor, for there are many serpentinic factors, that act in

different ways and combinations depending on local conditions".

Proctor (1971) showed that Agrostis species growing in serpentine soils take up an excess of magnesium over calcium, but this excess is not obligatory for their survival. He showed that Agrostis species had a high tolerance of magnesium The importance of nickel and chromium was also studied. Grasses on serpentine soils were more tolerant to nickel than those grown on more calcarious soil. Proctor found that oats grown in a serpentine soil had a very large excess of magnesium and he considered that the cause of toxicity of this soil was primarily due to high magnesium levels in the presence of low calcium levels. He suggested that ecotype adaptation to this toxicity was widespread in British and Swedish serpentine soils.

Studies on Japanese serpentine soils have also been made. Takagishi et al (1974) have studied the abnormal features of mulberry plants growing on soils derived from serpentine.

Wiltshire (1973) studied the yields and the uptake and distribution of nickel between roots and shoots, measured in relation to changes in availability of nickel from soils resulting from fertilization with nitrates. Fertilization increased crop yields with nitrate fertilization decreasing the nickel content of shoots more than did the ammonium ion. The concentration of nickel in roots was greater in all treatments.

Lyon et al (1971) studied trace elements in a New Zealand serpentine flora and concluded that no universal mechanism could be applied to explain plant survival on a New Zealand serpentine soil, but

differences were found between species in their ability to accumulate or exclude the various elements.

Ernst (1972) has undertaken ecophysiological studies of some heavy metal plants from South Central Africa, and found that the uptake of heavy metals depended on their availability in the soil. The uptake of heavy metals is specific for each species and within one species, for different tissues. Extractions of tissues of various plant parts showed that the binding of heavy metals within the cell was specific to the metal.

Shkol'nik and Smirnov (1972) experimenting with sunflower, showed that high concentrations of nickel and chromium induced certain morphological changes, such as dwarfism. They advanced the hypothesis that one of the main causes of morphological variations enduced by nickel and chromium is the increased R Nase activity leading to the destruction of nucleoli playing an important role in the preparation of cell divisions.

Ritter-Stadnicha (1972) made comparative studies of cell sap contents throughout the vegetation period on a number of plant species which occur on serpentine and calcium-rich soils. There was a general increase of magnesium, calcium and total free acids and oxalates throughout the vegetative period. The amount of total acids was higher in serpentine plants and the production of organic acids appeared to be stimulated by magnesium accumulation. Some species appeared to control the uptake of calcium and magnesium whilst others accumulated them randomly. The author concluded that the ability of individual species to grow on serpentine soils was a result of their physiological constitution.

So far little work has been done on the actual form of heavy metals such as nickel in the plant or how and why it is transported within the plant. Reilly *et al* (1970) has studied the accumulation and binding of copper in *Becium homblii*, whilst Tiffin (1971) has looked at the translocation of nickel in xylem exudate of plants but has not identified the nickel carrier.

Severne and Brooks (1971) have reported a high nickel accumulating plant, *Hybanthus floribundus*, growing on Western Australian ultrabasics and since then others have been found in New Caledonia. (Jaffrè, Latham and Quantin, 1971).

Recently Kelly *et al* (1974) have made some preliminary observations on the ecology and plant chemistry of some nickel accumulating plants from New Caledonia, most of them members of the Hybanthus family. Their work indicated a low molecular weight complex of nickel with certain amounts present as nickel (II) aqueous ions.

Out of this literature review several points emerge:

1. The toxicity and characteristic vegetation is due to the chemical composition of the parent material.
2. In accounting for surpentinic floras not only the toxic factors of serpentine soils must be considered, but also factors such as physical characteristics, soil depth, pH, calcium/magnesium ratio, magnesium, calcium, copper and other elemental concentrations.
3. In assessing the degree of toxicity other soil characteristics influencing vegetation must also be taken into consideration.

4. The possible physiological roles that nickel and chromium may have in plants which accumulate them.

This thesis reports investigations on the serpentine area known as the 'Mineral Belt' in Nelson, New Zealand and endeavours to outline a model to account for the distribution of the flora in this area. The plant chemistries of some nickel accumulating plants from New Caledonia are also investigated.

In this study the following points are considered:

(i) The chemical composition of serpentine soils and some selected plants.
(ii) Factors influencing plant distributions.
(iii) Antagonistic and mutually-stimulating plant - plant and plant-soil elemental pairs.
(iv) Statistical comparison of endemic and non-endemic plant sites.
(v) The plant chemistry of nickel.

SECTION I.

THE PHYSICAL ENVIORNMENT OF THE DUN MOUNTAIN AND NEW CALEDONIAN SERPENTINE AREAS.

The term serpentine is usually used to include all the mineralogical varieties comprising serpentinites and having the general formula $X_6 Y_4 O_{10} (OH)_8$ where X = Mg (Ni, Co, Mn, Fe^{2+}, Zn, (Al), (Cr), (Ti)), and Y = Si (Al, Fe^{2+}). Whittaker and Zussman (1956) using x-ray diffraction studies, have clearly defined the serpentinite minerals. Ultramafic is applied to dunite, harzburgite, peridotite and others, and their alteration product, serpentinite; it is prefered by many to the term ultrabasics.

A. THE DUN MOUNTAIN SERPENTINE AREA

1. Location, Topography and climate

The Dun Mountain serpentine area is approximately 12 km south-east of Nelson City, South Island, New Zealand (Latit. 41° 20's, Longit. 173° 20'E), and is part of the belt of ultramafics and volcanics extending from D'Urville Island to Red Hills. The width of this belt, often called the "Mineral Belt" (Bell et al, 1911) is seldom more than two miles or less than one mile. Its greatest width is in fact near Dun Mountain. This area, first recognised by Hochstetter (1859), is a zone of boulder-strewn country overlooked by the extrusive mass of the Dun Mountain from which the olivine rock, Dunite, takes its name. Plate I-1 shows an aerial view of the area. Wooded Peak, from which samples were taken, is drained on the southern side by the headwaters of the Roding River and on

the eastern side by the south branch of the Maitai River. The area was once mined for chromite and the site has the distinction of having New Zealands first rail line. The route of the old line still provides access by four-wheel drive. The area studied is of moderate relief ranging from 730 to 950 metres above sea level. In the valleys and the coastal areas the climate is mild, but on the "Mineral-belt" it is cooler in more exposed areas. The annual rainfall is approximately 150 to 250 cm depending on the altitude. Frosts and snow are frequent in winter on the higher slopes. A description of the climate and weather of the Nelson region is described by de Lisle and Kerr (1965).

2. Geology

Several writers have given descriptions of the general area within which samples for this study were taken. Lauder (1965a, 1965b) has described the rocks near Dun Mountain, Bell et al (1911) the "Mineral Belt", Waterhouse (1959) and more recently Coleman (1966) have discussed the whole region along with other New Zealand serpentinites.

The ultramafic belt consists mainly of serpentinites in various stages of alteration from their parent dunites, peridotites and harzburgites (Bell et al, (1911). Dun Mountain itself is a cone of unaltered dunite, surrounded

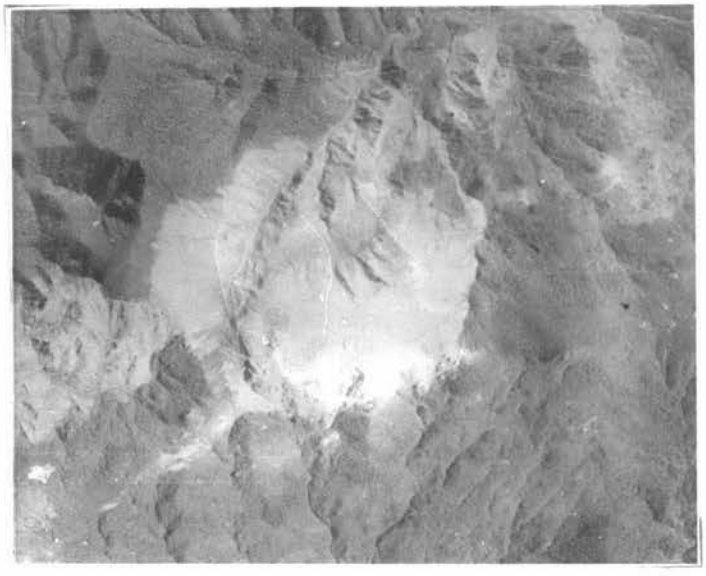


Plate I-1 Aerial view of Dun Mountain.

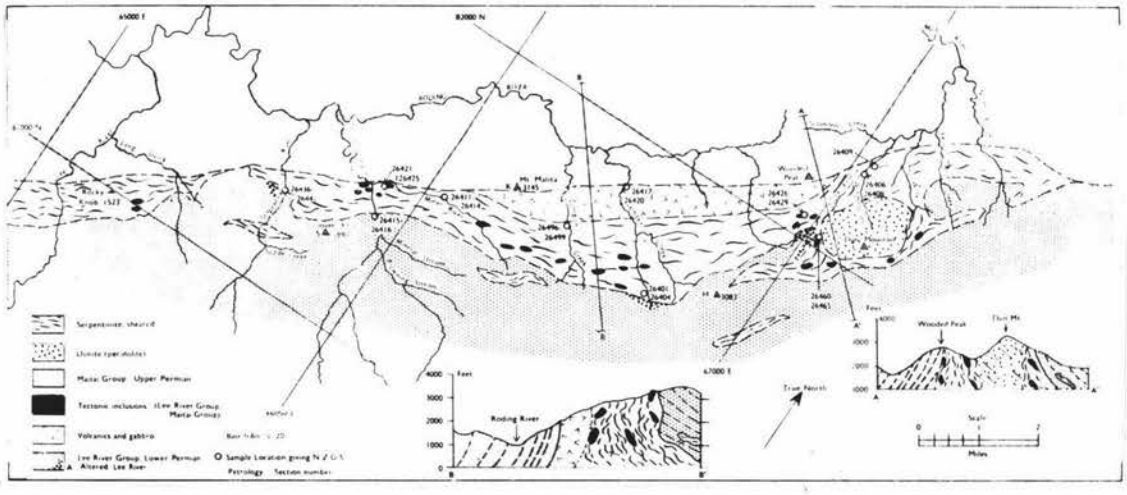


Fig.I-1 General geology of the "Mineral Belt".

by serpentinite (Battey, 1960). Coleman (1966) describes 'blocky' and 'sheared' serpentinites within the area. The ultramafic rocks are associated with sedimentary and volcanic rocks of the Permian, Te Anau and Maitai groups (Lauder, 1965) and are believed to be of Permian age (Grindley, 1958). Samples were taken from the southern slopes of Wooded Peak a short way below the serpentine-limestone boundary (Downslope movement has displaced this boundary considerably). Wooded Peak and surrounding bush-covered areas are underlain by sedimentary and volcanic rocks, mainly spilite, argillite and marble of Late Paleozoic age (Lauder, 1965a). Wooded Peak itself is composed of Wooded Peak Limestone (Waterhouse, 1959). The general geology of the area is shown in fig I-1.

Within the area can be seen outcrops of websterite which form oases amongst the more barren surfaces of the serpentinite. The soils of the area are shallow and poorly differentiated. A sample at about 6" depth usually represents the C horizon and is well penetrated by the root systems of all plants sampled. Occasional lenses of rodingite, a calcium-rich, basic igneous rock, are distributed through the region. These may be considered a more 'normal' substrate for plants.

Plate I-2 The serpentine-sedimentary boundary on Wooded Peak.

3. Vegetation

The stunted vegetation of the study area is in marked contrast to the luxuriant Nothofagus forest of the adjacent and more fertile Maitai state area on either side, a difference attributed by Cockayne (1928) and other early workers, to the high magnesium content of the 'Mineral-Belt' soils. The surrounding beech forests consists predominantly of Nothofagus solandri var. cliffortioides and Nothofagus fusca with Libocedrus bidwilli, Dacrydium bidwilli, phyllocladus alpinus, and several species of Coprosma. (Bell et al, 1911).

The plant associations of the serpentine are highly xerophytic and show remarkable adaptations to the existing conditions. The majority of the plants are shrubs showing the marked reduction in size and number of leaves, ramifying root systems and high development of cuticular protective tissue that are expected of plants growing under barren, adverse conditions. Typical of these characteristics are the endemics Pimelea suteri, Myosotis Monroi, Notothlaspe australe and Olearia serpentina. The area also supports species of Festuca and Poa and the ecotypes Hebe odora, Cassinia Vauvilliersii var. serpentina, Leptospermum scoparium and species of Coprosma and Celmisia.

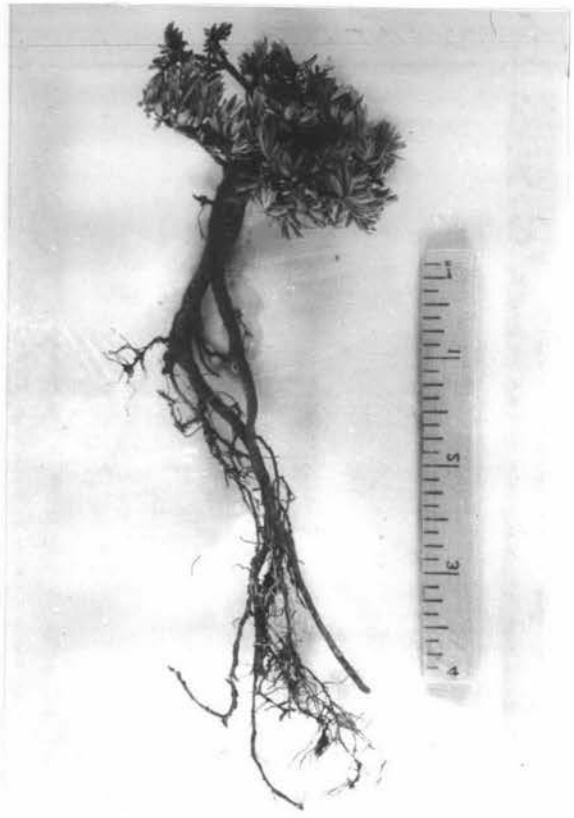
Species growing on both the sedimentary rock and the serpentine show remarkable differences in size and form. An example of this is the plant Pittosporum rigidum. When growing in the bush it is often 3 m high but on the serpentine it appears as a little cushion plant. Plate I.2. illustrates the abrupt change of vegetation type at the boundary of serpentine and sedimentary rocks. A transect was taken across this boundary for study (See Section III).

The plants selected for study were the two known endemics; Myosotis monroi Cheesem. (Boraginac), and Pimelea suteri Kirk (Thymelac); and the three most common non-endemic species; Cassinia vauvilliersii (Homb. et Jacq.) Hook.f. var serpentina Ckn. et Allan (Compos.), Hebe odora (Hook.f.) Ckn. (Scrophular) and Leptospermum scoparium J.R. et G. Forst. (Myrtac). Photographs of these are shown in Plates I-3.

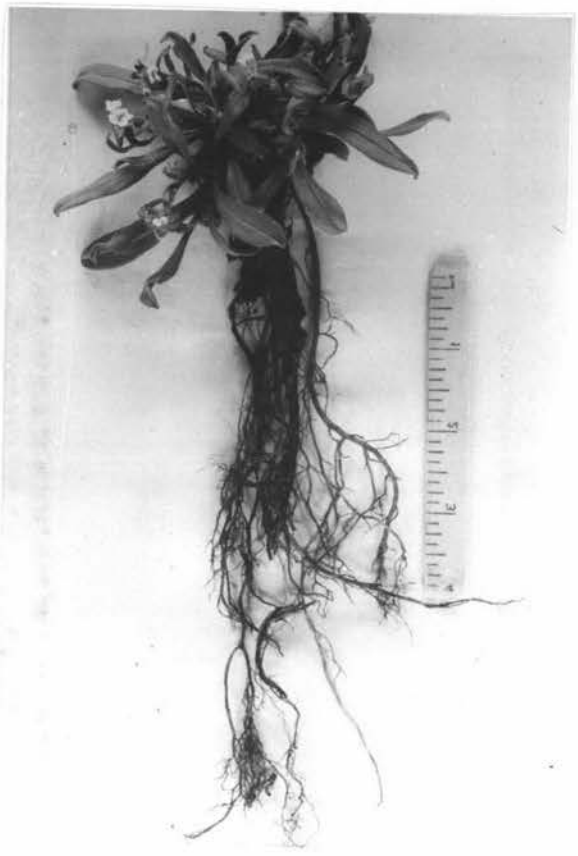
B. NEW CALEDONIA

1. Location, Topography and Climate

New Caledonia, a narrow, elongated island is some 400 km long by 40 km wide and lies roughly halfway between Australia and the Fijian islands. The plant samples came from the Boulinda Massif on the west coast of the island (Latit. 21° 19'S., Longit. 165° 6'E) at an altitude of 300 m



P. suteri



M. monroi

C. vauvilliesii

L. scoparium

Plate I-3 Species of plants studied.

(rainfall <180 cm) and the Plaine des Lacs (Latit. 22° 15' S. Longit. 166° 52' E) in the extreme south of the island in a zone of high rainfall (> 180 cm).

Most of the island is mountainous, plains being restricted to the valleys and to piedmont slopes found only on the western side.

2. Geology

The geology of New Caledonia is discribed in detail by Lillie and Brothers (1970). One third of the island consists of an ultramafic complex comprising large masses of peridotite and serpentinite. The southern massif, with an area of about 5,500 sq km, is the largest single ultramafic complex in the world. It is not an undifferentiated zone, and consists of chromiferous dunite, harzburgite, wehrlite and pyroxenolite as well as basic rocks, such as olivine gabbro, allivalite and norite. During weathering, silica and magnesia are dissolved and carried down from the mountains and iron, cobalt and chromium concentrate in the upper parts of the profile. Nickel first concentrates in the whole lateritic profile and then migrates slowly down the weathered profile (Baltzer et al 1967).

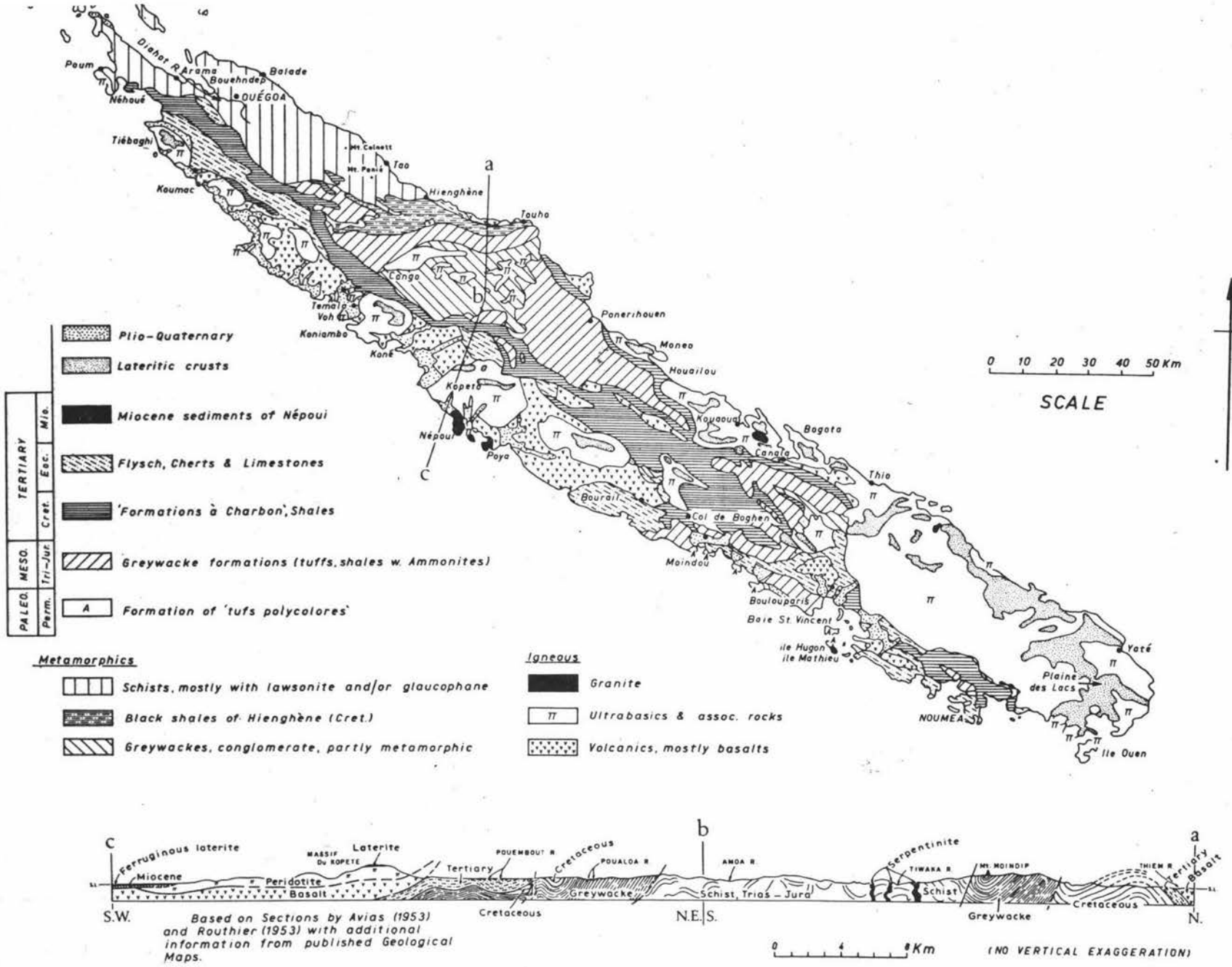


Fig. I-2 The geology of New Caledonia.

Routhier (1951) recognises two main types of ultramafic masses; the large bodies of peridotite and minor serpentinite, which make moderate mountains, and the smaller bodies of mostly serpentinite. He is of the opinion that the peridotite masses rest in other formations.

3. Vegetation

Jaffré et al (1971) and Jaffré (1973) have described in detail the vegetation of several ultrabasic areas; known to the locals as "Maquis des terrains miniers". The vegetation is shrubby and fairly open on the whole, but woody formations are well represented on iron rich soils, on favourable sites on talwegs, banks of rivers, sources of springs etc. The flora which is quite unique is adapted to survive under strong nutrient imbalances, high toxic element concentrations and an unfavourable calcium/magnesium ratio. These factors have combined to yield a very characteristic vegetation. (Jaffré, 1969, P. Quantin 1969).

The plant selected for this work, Homalium kanaliense, is found on hydromorphic, indurated, residual iron-rich soils with an upper gravelly horizon, on plateaus of medium altitude (500 m). It is often found in open, herbacious formations with Fristania quillainii, a dominant

species. Other species characteristic of this vegetation found along with H. kanaliense are Lophoschoenus stagnalis, L. comosus, Xanthostemon aurantiacum, Grevillea gillevrayi, Phyllanthus aeneus and Movria artensis.

SECTION II.

ANALYTICAL TECHNIQUES.

A. PREPARATION OF PLANTS AND SOILS FOR ANALYSIS

1. Sampling

Plants were located in a random manner by searching overall in an area of approximately 1 sq km. The area was representative, both topographically and geologically, of the whole ultrabasic region. The non-endemics were well represented but the endemics, particularly *P. suteri*, were rare. Therefore every observed speciman of *P. suteri* was sampled, the whole plant and root system being uplifted, together with about 100 g of soil taken from the C horizon below each plant. About 75% of all *M. monroi* encountered were sampled in the same way. About 10 g of leaf material was taken from each non-endemic sampled along with approximately 100 g of soil. Samples were taken in the month of May and seasonal effects were not considered to be of any significance in this project.

2. Ashing Procedure

Soil samples from the field were air dried for 48 hours and sieved through 100-mesh (150 μ) nylon. Organic material was decomposed by ashing in a muffle furnace at 450°C. Samples collected by Lyon (1969) were used for a preliminary survey but these differed from the author's own samples in that they were sieved to 40-mesh size before ashing and resieved to 100-mesh.

Air dried, minus 150 μ soil was used for soil extractions, and ashed fines for determining the total amount by atomic absorption.

Plant samples were carefully washed in running water, and then distilled water, and dried at 80°C. Fresh P. suteri leaves were put aside for freeze-drying. About 8 g of freeze-dried material was obtained, representing leaf material from 25 specimens. This material was kept for later work in plant chemistry. Enough plant material was ashed at 450°C to obtain at least 0.1 g of ash. At 450°C none of the elements determined in this study was lost in any significant amount. Weights of ash were recorded.

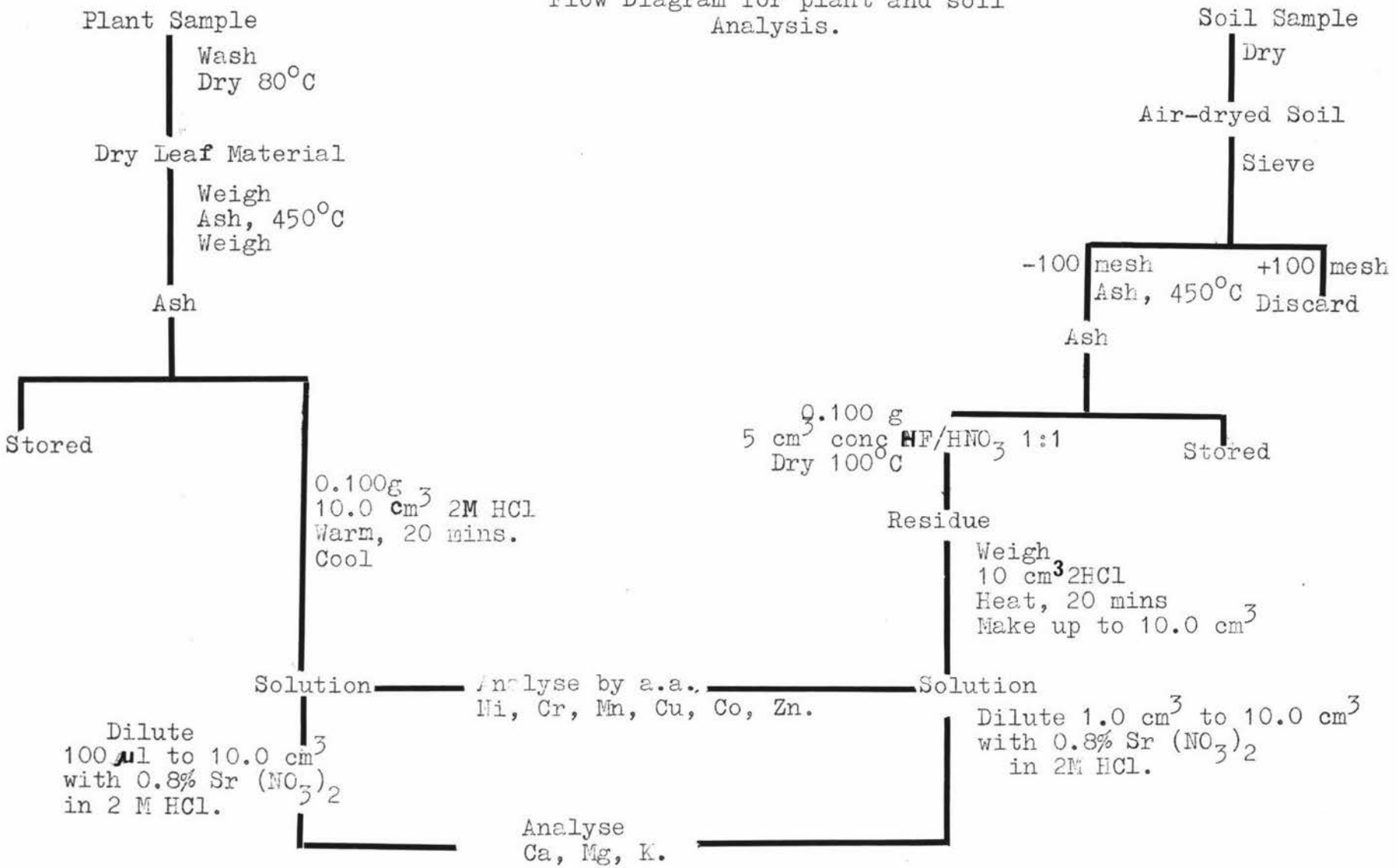
3. Dissolution

The method used for dissolving silicates was one similar to those described by Belt (1967) and Langmyhr and Paus (1968). Small samples of ignited soil (0.10 g) were weighed accurately into 50 cm^3 squat polypropylene beakers and digested with 5 cm^3 of a 1:1 mixture of concentrated hydrofluoric and nitric acids (1 cm^3 of 70% perchloric acid was also added as initial work gave low calcium results, and it was thought that small amounts of the very insoluble calcium fluoride may have been forming). The beakers were suspended in a water bath and the solution taken to dryness. The residues

were redissolved in approximately 8 cm^3 of 2M hydrochloric acid prepared from redistilled (5M) acid. After a further heating period of about 30 minutes, the solutions were washed into 10 cm^3 graduated polypropylene vials, made up to the 10 cm^3 mark with 2M HCl and stoppered. The solutions were then ready for analysis.

Plant samples (0.1 g of ash) were dissolved directly into 2M hydrochloric acid and heated for 30 minutes. These solutions were made up to 10 cm^3 in graduated polypropylene vials. For well-ashed material this method of dissolution was quite adequate. The 100 times dilution that this procedure gave, was satisfactory to bring all the trace elements into the analytical range desired, but further dilution was necessary for calcium, magnesium and potassium. The flow chart of fig II-1 shows the general procedure undertaken for all plant and soil analyses.

FIG. II-1.
Flow Diagram for plant and soil Analysis.



B. ANALYTICAL METHODS

1. Atomic absorption spectrometry

The Varian 'Techtron model A.A.5 atomic absorption spectrophotometer' was used for determining various elements under the conditions given in Table II-1.

Standards were made from analytical grade (Anala R) chemicals. Impurities at the concentration levels used, were not significant. Standards and sample dilutions for calcium and magnesium were made with 2M hydrochloric acid containing 0.8% Sr $(NO_3)_2$. Calcium forms refractory aluminates so a relatively hot flame was used to help overcome this effect. Phosphates, sulphates and silicates may also cause depression of calcium emission. Therefore the addition of a spectral buffer, very pure Sr $(NO_3)_2$, helped overcome these effects (Dinnin, 1960). The much analysed basic rock, W-1, was used for a reference standard (see Section II - C-3).

A combination standard was decided upon for all the trace elements, as these were all present in the plant and soil samples analysed. A combination standard would therefore be nearer the composition of the samples and would help reduce any matrix effects. Standards were initially made up in 1000 ppm concentrations. These stock standards were used for all work done throughout the 18 month period.

Standards were stored in polyethylene bottles. Losses or gains from the walls of these containers of trace elements were not significant, even for solutions as low as 2 ppm stored for long periods. These observations were supported later by the work of Struempler (1973) on various types of surfaces.

It may be noticed that in table II-1 there are two lines quoted for nickel. The 2320 line is the most strongly absorbing but is very close to the Ni^{+} ion line at 2319.9 and this causes problems of linearity of the calibration curve. (Mavrodineanu, ed,1970). When a hollow cathode lamp is used the less sensitive 3414 line was therefore used, and was satisfactory for most samples due to the high concentrations of nickel. The most sensitive line was used for all other elements and good calibration curves were obtained. A hydrogen lamp was used to correct for molecular absorption and light scattering effects. These were minimal in most samples.

TABLE II-1

INSTRUMENTAL CONDITIONS

Element	Wavelength (nm)	Lamp Current (MA)	Slit Width ( )	Sensitivity+ (ppm)	Flame Character	Standards (p.p.m. in 2M HCl)
Ni	232.0	5	170	0.05	Air-C_2H_2 Oxidizing	2,5,10,20,40 Comb*
	341.5	8	200	0.26		
Cr	357.9	5	100	0.055	Air-C_2H_2 reducing	1,2,5,10 Comb.
Co	240.7	5	150	0.053	Air-C_2H_2 Oxidizing	1,2,5,10 Comb.
Cu	324.7	3	100	0.04	Air-C_2H_2 Oxidizing	1,2,5,10 Comb.
Zn	213.9	5	250	0.009	Air-C_2H_2 Oxidizing	5,10,20,40 Comb.
Mn	279.5	5	200	0.021	Air-C_2H_2 Oxidizing	5,10,20,40 Comb.
Sr	460.7	10	250	0.041	N_2O-C_2H_2 reducing	1,2,5,10
Ca	422.7	5	300	0.013	Air-C_2H_2 reducing	2,5,10,20,40 with 0.8% $Sr(NO_3)_2$
Mg	285.2	5	200	0.003	Air-C_2H_2 reducing	2,5,10,20,40 with 0.8% Sr (NO_3)

+ From "Hollow Cathode lamp data" varion techtron, 1973

* A combination standard containing Ni,Cr,Co,Cu,Zn,Mn,Fe,Pb,Cd.

2. Colorimetry

Phosphorous in plants and soils was determined by forming a phospho-molybdate complex similar to that in the molybdenum blue method given by Stanton (1966). The reagent used was a mixture of 2M sulphuric acid, 0.025M ammonium molybdate, 0.1M ascorbic acid (freshly made each day) and 0.01M antimony potassium tartrate. These were made up as required in the proportions, 500 : 150 : 100 : 50. Aliquot size was such that each contained less than 40 µg phosphorous. A Unicam SP 1800 u.v. spectrophotometer with a slit width of 0.2mm was used to read the absorbance at 712nm and compared with a series of standards. It was found in this method that pH was critical and as the original solutions were in 2M hydrochloric acid they were neutralized with sodium hydroxide using p-nitro-phenol indicator.

3. Flame Photometry

Potassium was analysed, in the same solutions used in determining calcium and magnesium content, with a Gallenkamp flame photometer.

4. Chromatography

Various techniques in chromatography were employed in making some preliminary observations and deductions on the plant chemistry of some nickel

accumulators with emphasis on the chemistry of nickel in Homalium Kanaliense (New Caledonia) and Pimelea suteri (Dun Mountain)

(i) High-voltage paper electrophoresis

This was used to help in elucidating amino-acid patterns and the behaviour of nickel from various extracts of the plants studied. A "Savant"-type apparatus constructed in the department was used. This consisted of a glass tank comprising a lower buffer layer overlain with a water-cooled low flash point, petroleum spirit. The tank could accommodate 46 cm x 57 cm Whatman No. 1 and 3 MM chromatography paper. The papers were hung vertically from the upper buffer layer through the white spirit into the lower buffer layer and a voltage of 3 Kv applied for 40 minutes. Buffers of pH 2.1, 3.5 and 6.5 were used; that at pH 2.1 was a formic acid, acetic acid, water buffer (100 : 400 : 4500); at pH 3.5, pyridine, acetic acid, water (25 : 250 : 4610), and the buffer at pH 6.5 was pyridine, acetic acid, water in the ratios, 500 : 20 : 4500. A ninhydrin, collidine reagent was used for developing amino acids and α-furil dioxime for locating nickel.

(ii) Gel filtration

Various size columns with different sephadex cellulose gels were experimented with to establish the conditions under which separation of any nickel complexes, from the components of plant extracts, could best be obtained based on M.W. Small columns (10 cm x 1 cm) were used in preliminary work and final separations were carried out on a larger column (60 cm x 2.5 cm) coupled to a "Radi Rac" fraction collector. Cellulose powders were swollen in distilled water for 24 hours before column packing. The column was then washed with 0.05M ammonium acetate and the void volume determined using dextran blue (MW 2000 , Exclusion limit for sephadex G-10 is 700). A weak cationic exchanger, Sephadex C-25 was also used. Nickel was located in the fractions collected by atomic absorption.

5. U.V., visible and infra red spectrophotometry

Nickel complexes absorb in the visible and into the infra-red regions of the spectrum. It was therefore thought that the use of u.v. - visible and infra-red spectrophotometry may be of assistance in this study.

C. STATISTICAL TREATMENT

1. Data Analysis

An I.B.M. 1620 II computor was used to calculate geometric means, standard deviations and Pearson Product - Moment correlation coefficients (r). All concentration data were transformed to logarithms because elemental concentrations in soils and vegetation tended to be distributed log-normally rather than normally. Data were generally expressed on an ash-weight basis, but where data are reported on a dry-weight basis, the conversion from ash-weight to dry-weight is given.

Multi-element discriminant analysis was carried out with the computor to determine the extent to which different groups can be distinguished from one another on the basis of a set of measured properties. The measured variables were the concentrations of elements in the soil surrounding each plant sampled. Due to the log-normal distribution of the soil elemental contents, the data were initially transformed logarithmically.

2. Accuracy and Precision of Data

The U.S. Geological Survey reference sample, W-1 (Fairbairn, 1951) a diabasic rock, was analysed for the elements determined in the soil survey. The standard was treated in the same way as the samples and analysed along with them.

Results for the W-1 samples appear in Table II-2 and are compared with generally-accepted values. (Fleischer and Stevens, 1962 and Fleischer, 1965). These are based on the arithmetric mean of all determinations reported up until that time. As the determination of elements in silicate rocks is difficult (Taylor and Kolbe, 1964), the precision and accuracy obtained for all elements except zinc is seen to be satisfactory. (Contamination from many sources was found during the course of work to be a major problem for zinc. Zinc is particularly prone to leaching from glassware and polypropylene containers and although blanks were run this could not be entirely accounted for). In all cases, excepting zinc, the atomic absorption results were within or just outside the standard deviation of the best value (Fleischer 1965).

TABLE II-2 W-1 ANALYSES

	Element	No. of analyses 1	2	3	4	Mean	r.s.d.	s.d.	m.e.	Accepted Value *	Fletcher Timperley + ++
p.p.m	Ni	100	100	115	80	98.75	12.59	12.44	18.75	80	99.1+
	Cr	140	125	125	120	127.50	5.88	7.50	7.50	120	135.8++
	Co	70	70	60	62	65.50	6.95	4.55	13.50	52	73.7+
	Cu	125	115	115	110	116.25	4.68	5.44	6.25	110	117.9+
	Zn	142	126	122	92	120.50	15.00	18.07	38.50	82	97.6+
	Mn	1400	1450	1400	1200	1362.5	7.04	96.01	62.50	1300	1530 ++
%	Ca	7.40	7.40	7.00	7.00	7.20	2.77	0.200	-0.63	7.83	7.50++
	Mg	4.75	4.00	4.00	4.00	4.19	7.75	0.320	0.19	4.00	3.88++
	K	0.50	0.50	0.48	0.55	0.51	5.07	0.025	-0.02	0.53	0.512++

* Ahrens and Fleischer (1960).

\+ Fletcher (1970), on solutions diluted 0.100 g to 10.0 cm^3

++ Timperley Ph.D Thesis. Results from Varian A.A.5.

SECTION III

SOIL FACTORS INFLUENCING A SERPENTINE FLORA.

A. GEOBOTANICAL STUDY

A small cross-section of the serpentine-sedimentary boundary was examined in order to understand more fully the vegetational changes involved, and to verify on a more quantitative basis the outstanding changes that occurred, which were so obvious to the eye (Plate I-2).

1. Sampling

A short transect of 48 metres was taken across the boundary, on the slopes of Wooded Peak. Unfortunately it was difficult to find a relatively flat area, so the actual limestone-serpentinite boundary is somewhat disguised by a certain degree of downslope movement of surface material from the limestone area. Beginning on the upslope sedimentary side plant species were counted in 6 metre quadrats, and specimens taken for identification. Soil samples were taken from below the humus layer every 3 metres. These were prepared for analysis as outlined in the previous section.

2. Soil Geochemistry

Each soil sample along the transect was analysed by atomic absorption for several elements. Fig III-1 shows the trends of nickel, chromium, magnesium and calcium. Manganese decreased on going from the sedimentary substrate on to the serpentine, and cobalt increased as was expected. Copper and zinc concentrations remained fairly constant over the whole transect. Fig III-1 shows the inverse relationship between the nickel and calcium, and the calcium and magnesium concentrations. Downslope creep somewhat distorted the concentration trends of the elements.

3. Plant Distributions

Fig III-1 shows the distribution of herbaceous and woody shrubs, and larger trees, along the transect. The Nothofagus forest and associated tree species stops at the 24 metre mark and a low shrubby and herbaceous vegetation takes command, of which the dominant species are Leptospermum scoparium, Cassinia vauvilliersii var. serpentina, Hebe odora and species of Dracophyllum. Of these only H. odora extended to any extent on to the sedimentary substrate, the others presumably being unable to compete with the dense vegetation growth and preferring the more open serpentinic area on which they have successfully adapted, to survive the harsher elemental conditions. The Dracophyllum genus showed a succession of species along the whole of the transect. Four species in all were differentiated.

4. Discussion

The visible boundary appears at the 24 m mark and this is shown by fig III-1. The trough in the nickel, chromium and magnesium values at 18 and 21 metres probably represents some creep of sedimenary material across the true serpentine-sedimentary boundary due to downslope movement. This has enabled some species to extend further onto the serpentine. There was a preponderance of N. solandri and D. filifolium in this quadrat. H. odora seemed to grow both on and off the serpentine area with equal facility, but the ubiquitous L. scoparium and the mountain cotton-wood, c. vauvilliersii, may have developed ecotypes which have been able to survive on the high nickel, magnesium soils of the serpentine.

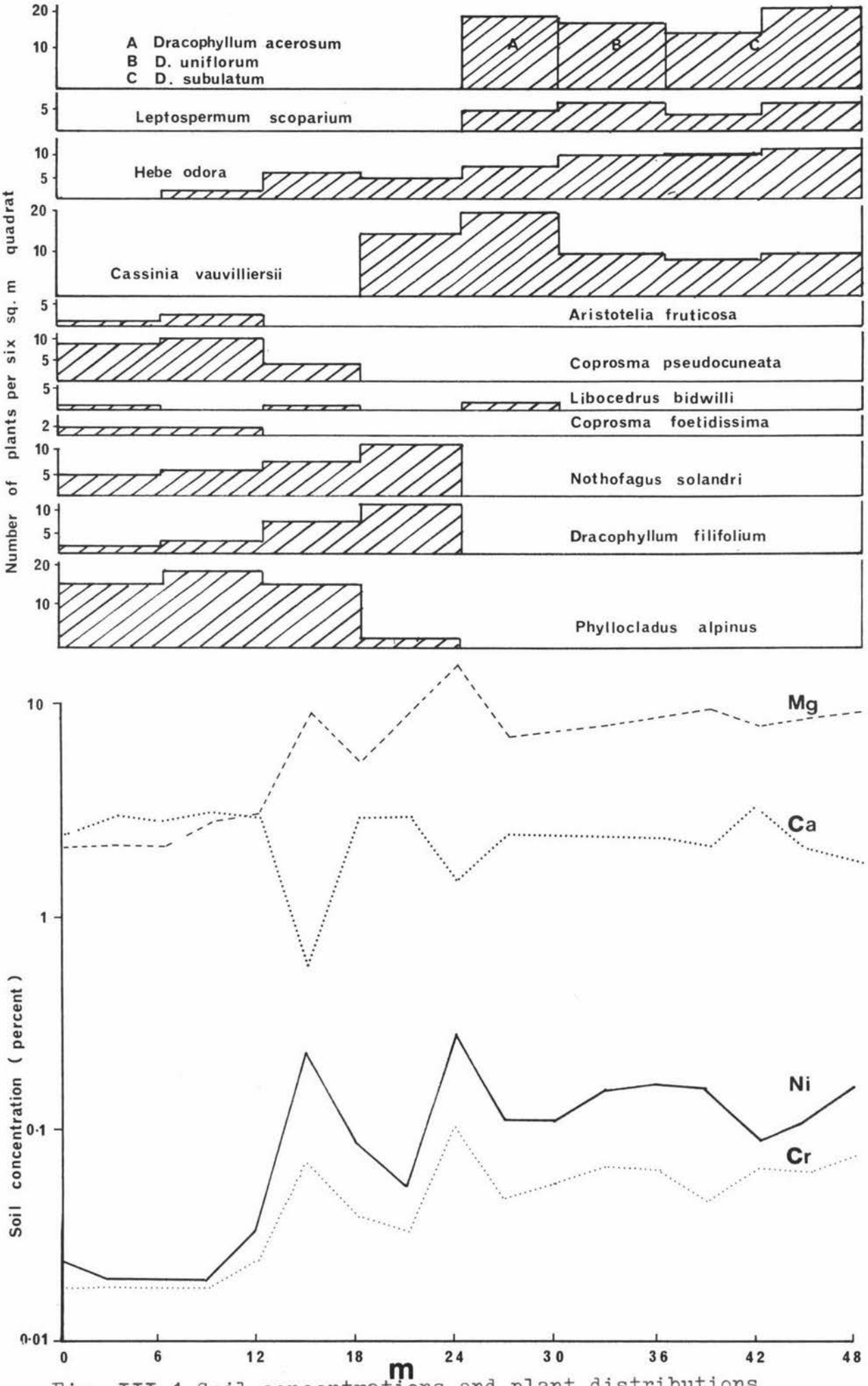


Fig. III-1 Soil concentrations and plant distributions across sedimentary-serpentine boundarys.

Forest plants, such as the gymnosperms, beeches and coprosmas seem unable to survive on serpentine. They are obviously able to tolerate the xerophytic alpine environment for they are seen to grow on the oases of non-serpentinite material within the "Mineral-Belt". Therefore it must be some soil factor which restricts their habitat, and which is responsible for the sharp boundary 'line' between the two very contrasting floras. The nature of the dominating soil factors, if any, was the aim of further work in this study. The three species L. scoparium, H. odora and C. vauvilliersii var serpentina were selected for the main sampling study because of their ubiquitous nature and because they are readily identified in the field. The two endemics, M. monroi and P. suteri were selected because they might be expected to provide some information, or evidence, for their refined distribution.

B. PLANT AND SOIL SURVEY

This survey was designed to provide some insight into the role that the serpentine soil chemistry plays in dictating, if in fact it does, the type and distribution of the flora. The ultimate aim as to isolate any dominant factors that may prevail. Various soil and plant data were obtained on which several statistical methods were used.

1. Sampling

Collection of samples were carried out as described in section II-A-1. Table III-1 gives the number of each species collected on the 13th and 14th of May, 1973 from 115 sites along the serpentinized areas of Wooded Peak. The total area covered was approximately 1 sq kilometer.

Samples were well washed in running water and then distilled water. This was important because of the low-growing nature of many of the plants and the loose, broken surfaces of the soil.

TABLE III-1 Species Sampled

SPECIES	PLANT	SOIL
Endemics (E)		
P. suteri	26	26
M. monroi	29	29
Non-endemics (N)		
H. odora	18	18
L. scoparium	23	23
C. vauvilliersii var. serpentina	19	19

Although the numbers in each group were not at all large, they were thought to be sufficiently great to allow some reasonably valid statistical deductions to be made.

2. Soil Geochemistry

(i) Total Soil Element Concentrations

All soils for each plant species were analysed by atomic absorption spectroscopy, using the preparations and conditions outlined in Sections, II-A-2, II-A-3 and II-B-1, for nickel, chromium, cobalt, copper, zinc, manganese, magnesium and calcium.

Potassium was determined by flame photometry. In a preliminary survey, lithium, lead, phosphorous and strontium were also determined. The first three were found to be very invariable throughout the soil samples. Lead concentrations were 30-40 ppm, lithium from 10 to 15 ppm and phosphorous fluctuated slightly above and below 500 ppm. Strontium results were suspect due to various interference problems which could not be adequately resolved. These four elements were therefore not considered further.

Table III-2 shows the geometric means for the concentrations of the elements determined in soils from each of the five plant species. Geometric means were taken as the soil concentrations are known to be log-normally distributed. The higher concentrations of nickel and magnesium for soils from the endemic species (E), the high potassium content of P. suteri soils, and the lower copper concentration in the same soils are immediately obvious. The same information is given in fig III-2 together with the standard deviations. This further demonstrates the marked differences between the soils supporting endemics, and those supporting the three non-endemic species. For all elements except copper, the standard deviations are generally much lower for the "E" soils than for the "N" soils. Note particularly the very low standard deviations of nickel and magnesium for endemic-supporting soils. This seems to indicate their advanced adaptation to higher nickel and magnesium soils, and that they are restricted to these soils through competition. This is in marked contrast to H. odora which

is seen to grow equally well on all types of soil. C. vauvilliersii is not so well adapted and is restricted to soils with more favourable elemental conditions. The constancy of total calcium in all soil groups can be readily seen from this figure; the geometric means being especially constant. As may be expected, the toxic elements of nickel, chromium, cobalt and magnesium show the greatest variation over the nutrient elements except in the case of the endemic soils. Note the marked negative skewness of the nickel distribution in the soils bearing H. odora specimens. This correlated with other factors, (see plant element distributions, Fig III-4) which pointed to the exceptional geographical distribution with varied soil conditions, under which this plant is able to exist. The plot for magnesium distribution showed a positive skewness whilst the other elements showed approximate log-normality. In general H. odora, along with L. scoparium to a slightly lesser extent, showed variations in soil concentrations for nearly all the elements to a greater degree than the other plants. These two plants have, either highly developed exclusion mechanisms, or are able to withstand varied soil element conditions to a greater degree than the others. This point will be looked at in section III-3 where the concentrations of various elements in the plants themselves are analysed and the distributions shown.

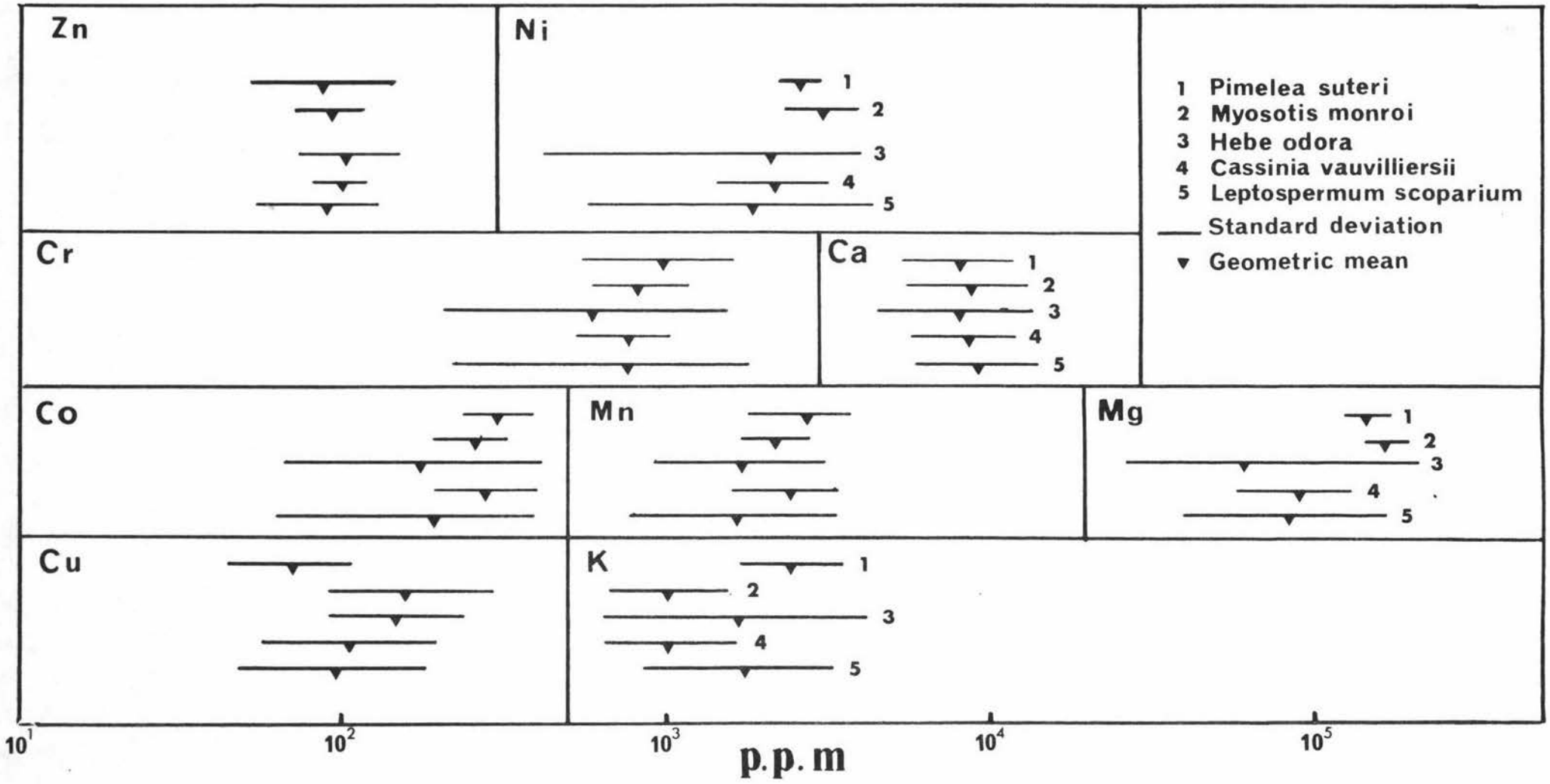


Fig. III-2 Elemental concentrations in five soil groups.

TABLE III-2

Mean (geometric) elemental concentrations in groups of serpentine soils collected from the base of five species of plants

		MEAN ELEMENTAL CONCENTRATIONS							
	No.	Ca (%)	Co (ppm)	Cr (ppm)	Cu (ppm)	K (ppm)	Mg (%)	Mn (ppm)	Ni (ppm)	Zn (ppm)
Group 1 (*P. suteri*)	26	0.82	292	926	69	2460	14.89	2630	2630	84
Group 2 (*M. monroi*)	29	0.87	252	811	155	1009	16.37	2133	3013	91
Group 3 (*H. odora*)	18	0.82	176	567	146	1637	7.55	1694	1517	103
Group 4 (*C. vauvilliersii*)	19	0.85	278	765	104	1030	9.14	2399	2163	97
Group 5 (*L. scoparium*)	23	0.90	194	756	94	1734	8.60	1656	1862	85

(ii) The Availability of Elements to Plants

The soil analyses showed that large total quantities of heavy metals are found in the serpentine soils of the "Mineral Belt". However in what form and quantity are these metals available to plants? From the plant species studied, it is seen that different elements are extracted from the soil by different plants to various degrees. (see fig III-4) These may be correlated with the "total" soil concentrations only to a limited degree. Although a thorough investigation of proportions available to plants in the soil would have been beneficial in this work, it is out of the scope of the present study, which has taken a more simplified route for a preliminary study only. However the next part of this section will try to assess the labile pool of ions available to P. suteri using various extractants.

Various extractants have been used by a number of workers in assessing the above quantity. Lopez (1973) showed that D.D.T.A.-$CaCl_2$ mixture removed a major proportion of the labile pool of micro-nutrients. Beyers and Hammond (1971) used 2% nitric acid, 1% E.D.T.A., 0.1M hydrochloric acid and 2.5% acetic acid in finding a relationship between copper uptake by barley seedlings, and various forms of extractable soil copper. Working with a serpentine soil, Halstead (1968) extracted available nickel by leaching with 1M ammonium acetate, or equilibrating the soil sample with 0.1M calcium chloride.

a. Method

In this brief study, several extractants were used on 2g samples of dried soils from the P. suteri sites. Each sample was shaken for 24 hours

with five times its weight of extractant. The resulting solution was then centrifuged at 5,000 rpm and the supernatant filtered. This was evaporated to dryness on a water bath, and the residue redissolved in 2M hydrochloric acid. Atomic absorption was used to analyse the samples. The results are reported in table III-3. The pH of the soils varied only slightly. A value of 6.5 being typical of most. The values are taken as an approximate guide to the quantity readily available to plants.

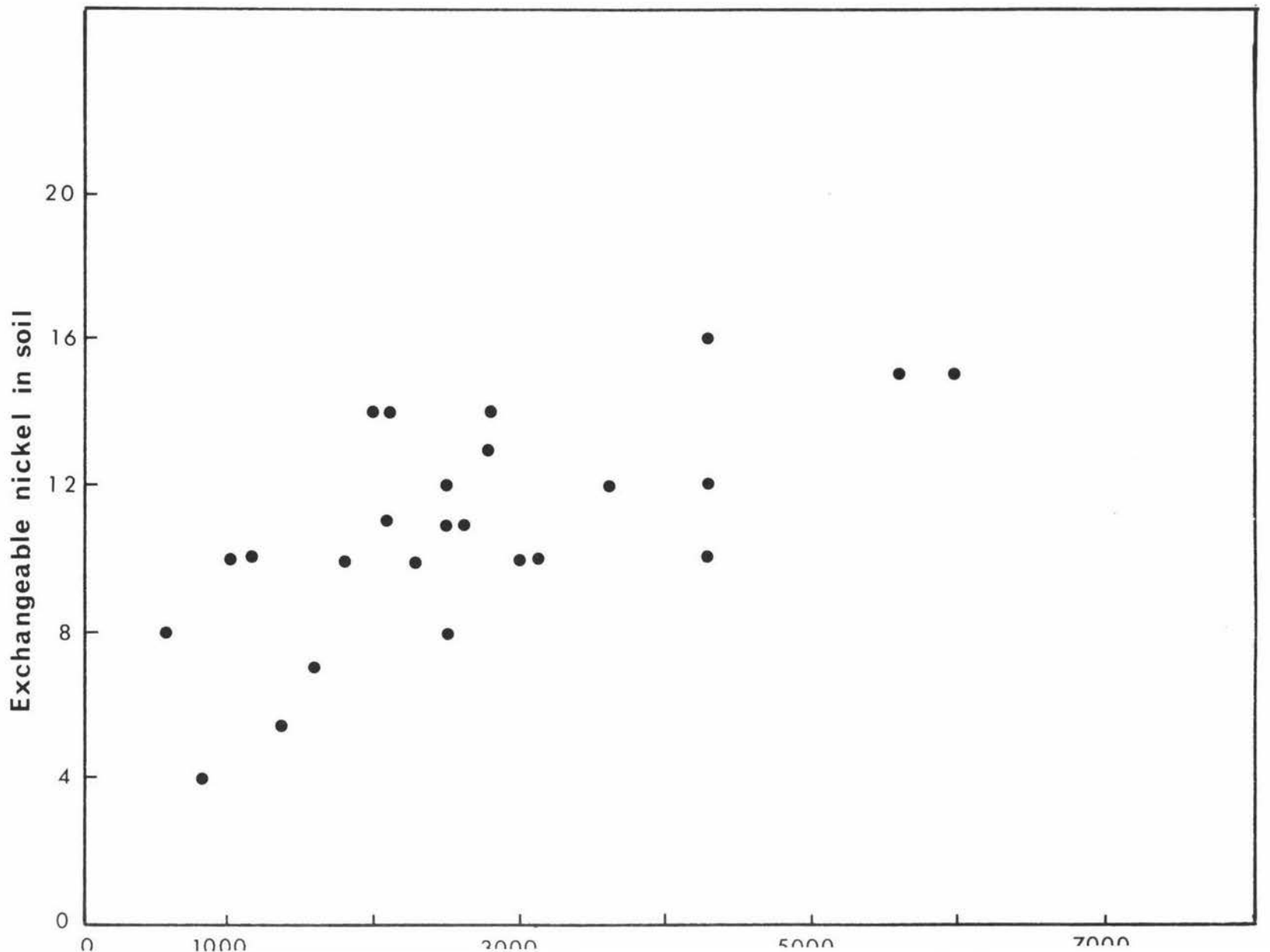
b. Results

A plot of nickel concentrations in the leaves of P. suteri against the amount of nickel extracted by 0.1M ammonium acetate (fig III-3) shows a fairly strong relationship. It is therefore reasonable to assume that the quantity of nickel thus extracted is representative of that available to the plant. The mean concentration of nickel extracted by 0.1M ammonium acetate is approximately 11 ppm, which is the level suggested by Scott and Mitchell (1943) as a possible toxic level. Some other serpentine soils show higher levels of extractable nickel. (Menezes de soqueira, 1968; Hunter and Vergnano 1952; Spence 1957 and Fernandez et al, 1965). Lyon (1967) showed that 2.5% acetic acid extracted a mean value of 132 ppm from soils of the mineral belt. This is nearer the values obtained in table III-3 for 0.2M hydrochloric acid and 0.1M E.D.T.A. Extraction of the soils from the other four plant groups was not considered and useful comparisons can not therefore be made, but it is obvious that P. suteri is able to extract larger amounts of nickel (table III-4), or that the other species have exclusion mechanisms of various degrees of effectiveness.

The low concentration of soluble chromium (even using 0.2M hydrochloric acid only 0.6% was extracted) probably bears some relation to the chromium in chlorides (Menezes de Sequeira, 1968). This is plausible as the majority of chromium exists as the very insoluble chromite, which is even resistant to quite strong acid attack. The amount extracted did not bear any relation to the total amount present in the soil. This was generally the case found for all the elements determined.

Available copper and zinc is relatively high, and is similar to other serpentine soils in Portugal (Menezes de Sequeira, 1968) and Spain (Fernandez et al 1965). Note the large quantity of cobalt extracted by 0.1M E.D.T.A.

However although exchangeable amounts of any cation may be estimated, it is almost impossible to determine what is plant available, as availability varies with species of plant, physical nature of soil, climate, microbial activity of soil, and many other factors (Schütte, 1964). Leaf analysis probably indicated best approximations to available amounts in the soil, although it did not indicate directly the plant's physiological status or requirements.


Exchangeable nickel in soil
20
16
12
8
4
0
0
1000
3000
5000
7000

Reagent used	Mean amount of element extracted (ppm).				
	Ni	Cr	Co	Cu	Zn
0.1M NH_4OAc	11	2	0.5	2	-
0.2M HCl	270	6	35	3	9
0.1M $CaCl_2$	10	2	2	2	5
H_2O	1.5	1	0.3	1	2
1% $ZnSO_4$	46	2	3	2	-
0.1M EDTA	180	3	61	6	9
TOTAL (26)	2630	926	292	69	84

TABLE III-3
Mean concentrations of metals extracted by various reagents from soils of P. suteri.

3. PLANT ANALYSIS RESULTS

Plants were analysed by atomic absorption using the conditions given in section II. Table III-4 shows the means of the elemental concentrations in the leaves of the five plant species. Results are given on a ash weight basis and a dry weight basis. The high ash weight of M. monroi reflects its different type to the small merophytic leaves of the other species; M. monroi being a herb whilst the others are woody stemmed and generally shrub-like in nature. This high ash weight means that M. monroi has high nickel, chromium, cobalt and manganese concentrations on a dry weight basis but often lower values in its ash than some of the other species. However in both cases it was first

noted that the nickel concentrations in the two endemic species are far higher than those for the non-endemics. This reflected the same situation as their corresponding soils. Due to the high magnesium content in the endemics, the calcium/ magnesium ratio was much lower (less than 1) than the non-endemics, where it was greater than 1, with L. scoparium attaining a value of 2.08.

The very low value of chromium in H. odora compared to the other species seemed to be of some significance, since, as mentioned previously, this plant was seen to grow on soils with a wide range of chromium concentrations. But as seen from fig III-4, the standard deviation of chromium in the leaves of this plant was very low. Thus, this may point to a very efficient exclusion mechanism by H. odora for this element, as it seemed to be very tolerant of high chromium levels. L. scoparium on the other hand showed much higher levels of chromium in its leaves, and this has been correlated by some workers with a high calcium level (Lyon, et al, 1971). Maximum and minimum concentrations found in this species for chromium were 650 ppm and 20 ppm respectively, but for both plants, the calcium levels were approximately equal. The author found no significant correlation between these two elements in the plant. However the case for M. monroi is interesting. Note the high chromium value in the leaves on a dry weight basis and also the surprisingly high calcium concentration (0.73% dry weight). (A very strong negative correlation between these two elements was also found; r:-0.6024).

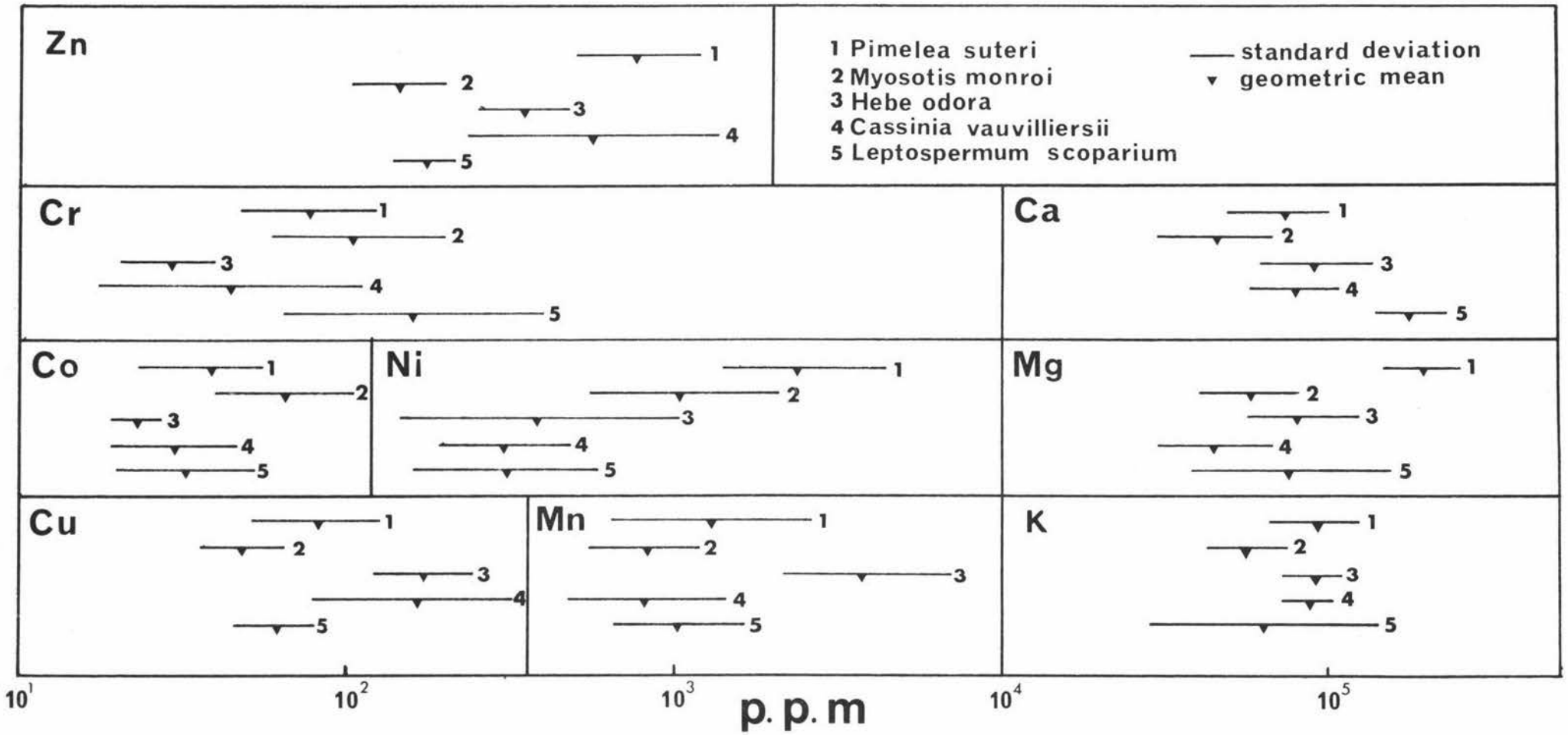


Fig. III-4 Elemental concentrations in five species of serpentine plants.

Fig III-4 shows, by graphical representation, the geometric means and the standard deviations of the elements analysed.

C. STATISTICAL EVALUATION

The data presented in the foregoing sections have been subjected to two major statistical treatments. Firstly, all the soil data were evaluated by a multivariate technique and discriminant functions obtained for five soil groups, and for the two major groups, namely the endemics and the non-endemics. Secondly all soil and plant data were evaluated to obtain Pearson correlation coefficients, and the significance of any highly correlated elemental pairs.

1. Elemental inter-relationships

Pearson correlation coefficients were obtained from log data for all the element-pair combinations using a Burroughs B6700 computor. These fell into three groups, intra-soil correlations, of element pairs, inter-soil-plant correlations and intra-plant correlations. With one or two exceptions, it can be seen from figs III-2 and III-4 that the elemental distributions are normal on a logarithmic basis. This is a requirement if meaningful correlation coefficients are to be obtained. Correlations were obtained on a ash weight basis although it has been seen that the percent ash weight of M. monroi differs significantly from the other four species, the variation in percent ash from sample to sample within each species was not particularly great. The correlations will therefore approximately reflect those obtained on a dry weight basis. Interelement correlation

TABLE III-4

Mean elemental concentrations in Leaves of Plants Sampled

Species	No.	% Ash	(ppm) Ni	Cr	Co	Cu	Zn	Mn	(%) Ca	Mg	K	Ca/Mg
Leaves-Ash												
P. sutcri	26	4.92	2389	76	39	82	756	1299	7.68	19.44	9.46	0.39
M. monroi	29	16.04	1049	107	65	48	141	813	4.60	5.93	5.75	0.76
H. odora	18	3.16	387	29	23	173	345	3774	9.27	8.37	9.36	1.10
C. vauvilliersii	19	3.95	300	44	30	163	556	805	8.11	4.53	9.05	1.60
L. scoparium	23	2.76	308	157	33	61	172	1003	17.70	7.74	6.44	2.08
Leaves-Dry												
P. Suteri	26	4.92	114	3.8	1.9	4.0	37.2	13.9	0.38	0.95	0.46	0.39
M. Monroi	29	16.04	168	17.1	10.4	7.6	22.5	130.0	0.73	0.94	0.92	0.76
H. odora	18	3.16	11.6	0.87	0.7	5.3	10.3	113.2	0.27	0.25	0.28	1.10
C. vauvilliersii	19	3.95	12.0	1.8	1.2	6.5	22.2	32.2	0.32	0.18	0.36	1.60
L. scoparium	23	2.76	8.6	4.4	1.0	1.7	4.8	28.1	0.49	0.22	0.18	2.08

coefficients in the soils, plants and between soils and plants are shown in figs III-5a, III-5b and III-5c. Only correlations that are significant above the 99.0% probability level are shown.

(i) Elements in Soils

The correlations for the five plant groups are illustrated in Fig III-5a, b and c. L. scoparium and H. odora have the largest number of highly significant correlations which seems to reflect their more universal distribution. The most significant correlation was between nickel and cobalt ($r = 0.92$ for the soils of L. scoparium and 0.96 for those of H. odora). In all the non-endemics there were also strong correlations between the elements cobalt, manganese and zinc. Generally, the correlation between elements was very similar in L. scoparium and H. odora. Of some interest was the strong negative correlations between nickel and potassium, and potassium and cobalt which were common to M. monroi and H. odora. Mentioned in the previous section was the relationship between calcium and chromium in M. monroi. It was noticed from Fig III-c that there was a positive correlation between these two elements in the soils supporting this plant, but negative in the plant leaves as pointed out later.

It was expected that there might be some significant relationship between calcium and magnesium in the soils; that is, when magnesium is high calcium is low. These elements are found to be negatively correlated but are at the most only marginally significant. (C. vauvilliersii soils had a correlation coefficient of -0.32 at a probability level of 0.087). More significant correlations may have been found if exchangeable cations as well as total quantities, had been measured.

(ii) Elements in Plants

The most significant point to be seen from the illustrations reflecting the inter element relationships in the plant leaves, is the number of highly correlated elements involving nickel in the two endemic species. P. suteri shows no significant negative or antagonistic pairs but M. monroi has a number of highly correlated pairs. Note the antagonistic chromium, calcium pair mentioned earlier. Other very significant negative correlations are between nickel and calcium; nickel and potassium, and with the calcium/magnesium quotient. High magnesium in the plant is associated with low calcium, this relationship is not obvious for the other species.

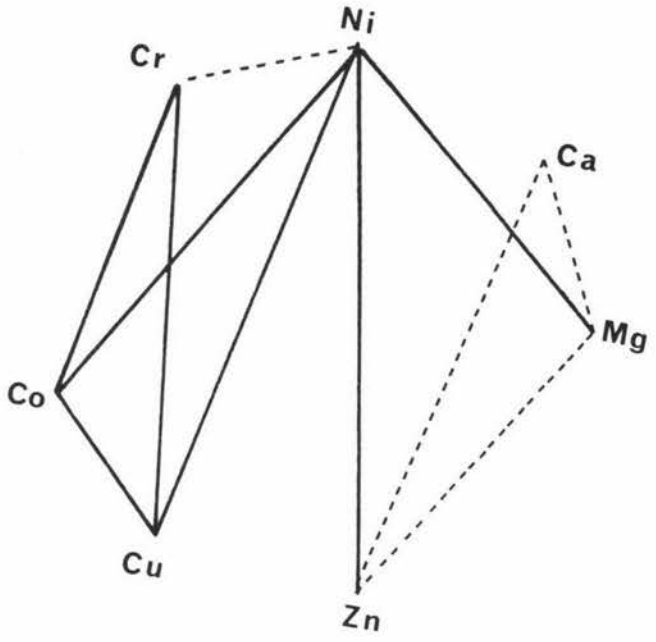
It may be expected in some cases that certain relationships that exist in the soil may also be reflected in the plant. Looking at those correlations for M. monroi it can be seen that nickel is heavily correlated with potassium (negatively) in both the soil and the plant. Nickel and calcium also show a similar trend. Chromium and calcium are positively correlated in the soil (0.48) but strongly antagonistic in the plant. There are other pairs also that reflect the relationships that exist in the soil. For instance nickel and cobalt in H. odora; chromium and cobalt, magnesium and zinc in P. suteri; cobalt and zinc in C. vauvilliersii, and nickel and chromium in L. scoparium. The latter plant also shows another inverse pair. Magnesium is positively correlated with potassium (0.75) in the leaves but negatively correlated in the soil (-0.59). H. odora and M. monroi are the only two plants to show the negative calcium-magnesium elemental pair to be significantly correlated.

(It seems that there are many similarities between these two plants even though M. monroi is endemic to the region whilst H. odora has a much more widespread distribution). In contrast to H. odora and M. monroi, P. suteri shows a positive relationship between calcium and magnesium. That is, it seems to accumulate calcium and magnesium in the same relative amounts.

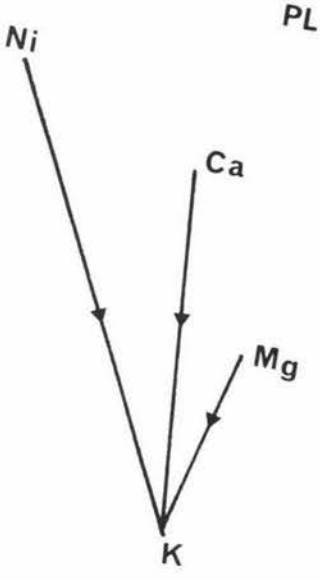
(iii) Plant-soil Correlations

From the calculation of these correlations it was possible to determine whether elements in the plants bore any relation to those in the soil, and, also to determine whether any elements in the plants bore a relation to the amount of other elements in the soil.

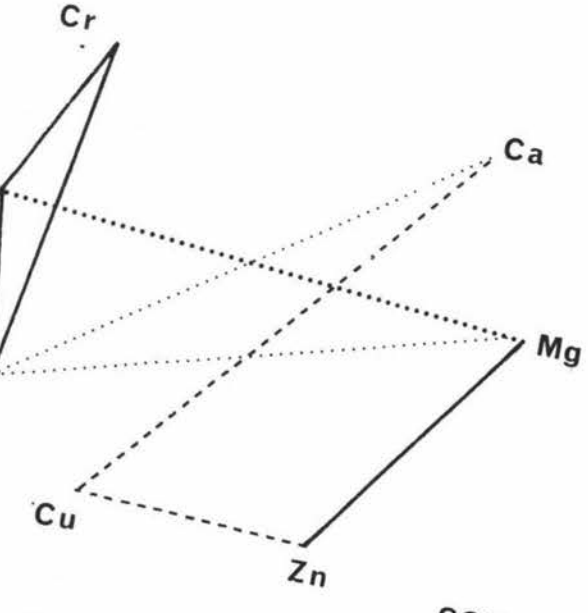
Again H. odora and L. scoparium showed the greatest number of significant correlations as for the soils. Correlations between a particular element in the plant and its soil are shown in table III-5. Other inter-element relationships are shown in figs III-6a, b and c.



PLANT- PLANT



PLANT - SOIL



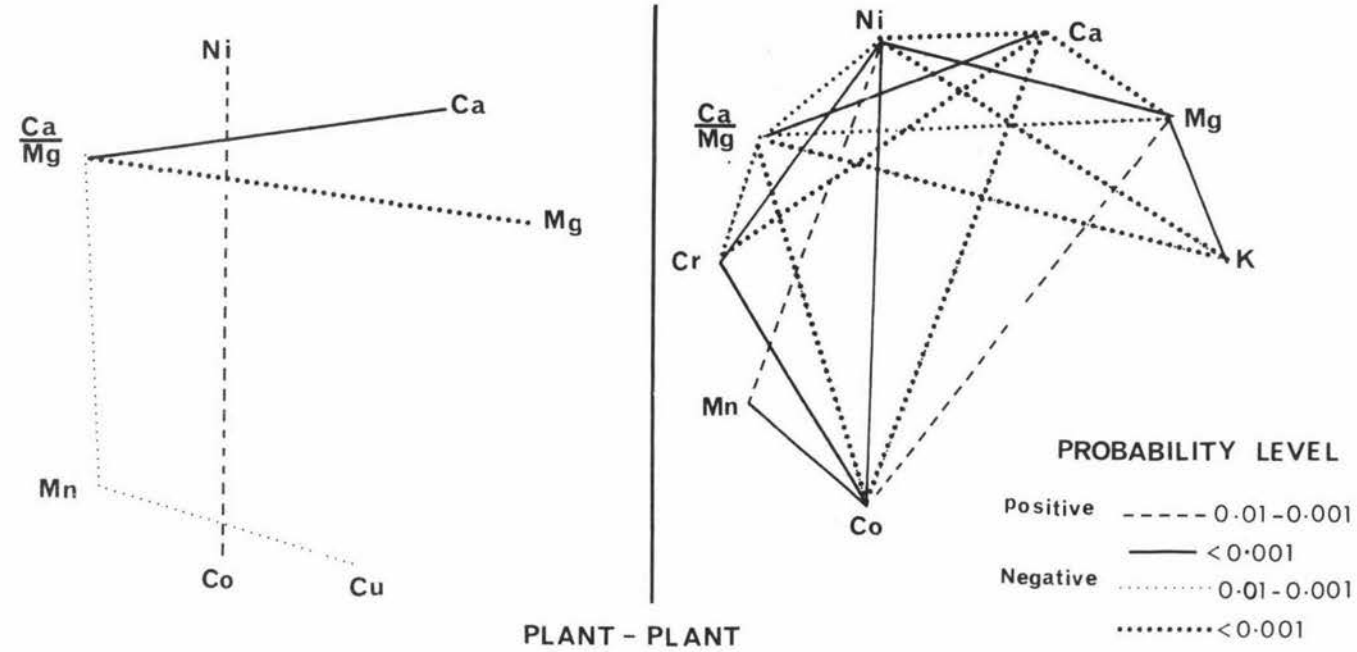
SOIL - SOIL

PROBABILITY LEVEL

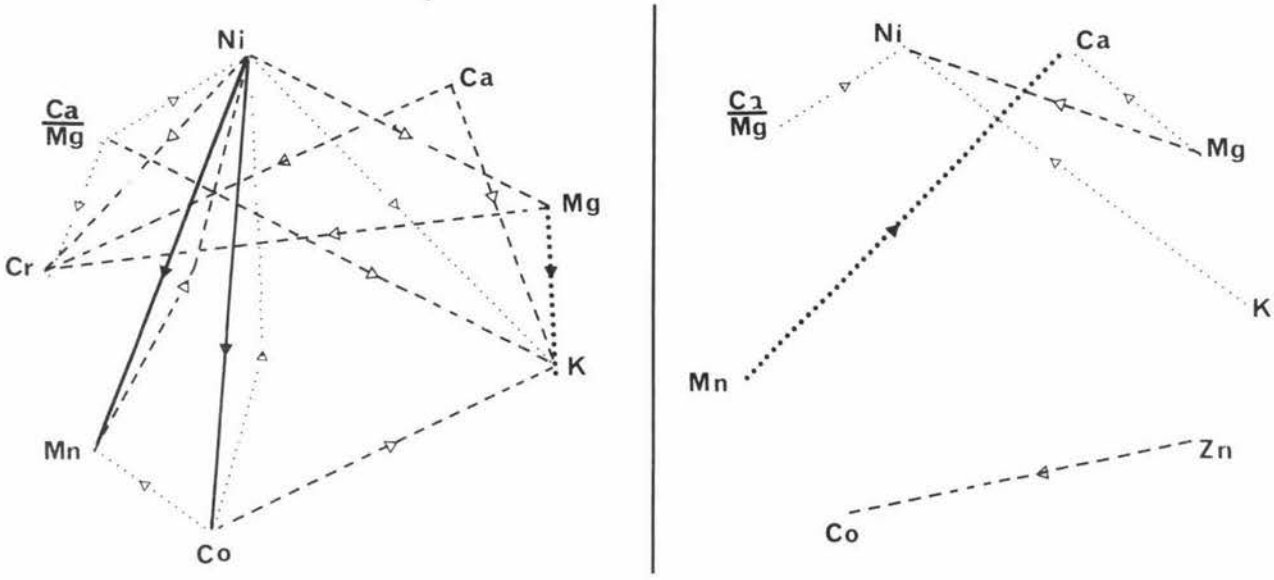
Positive
- - - - 0·01 - 0·001
——— < 0·001

Negative
····· 0·01 - 0·001
····· < 0·001

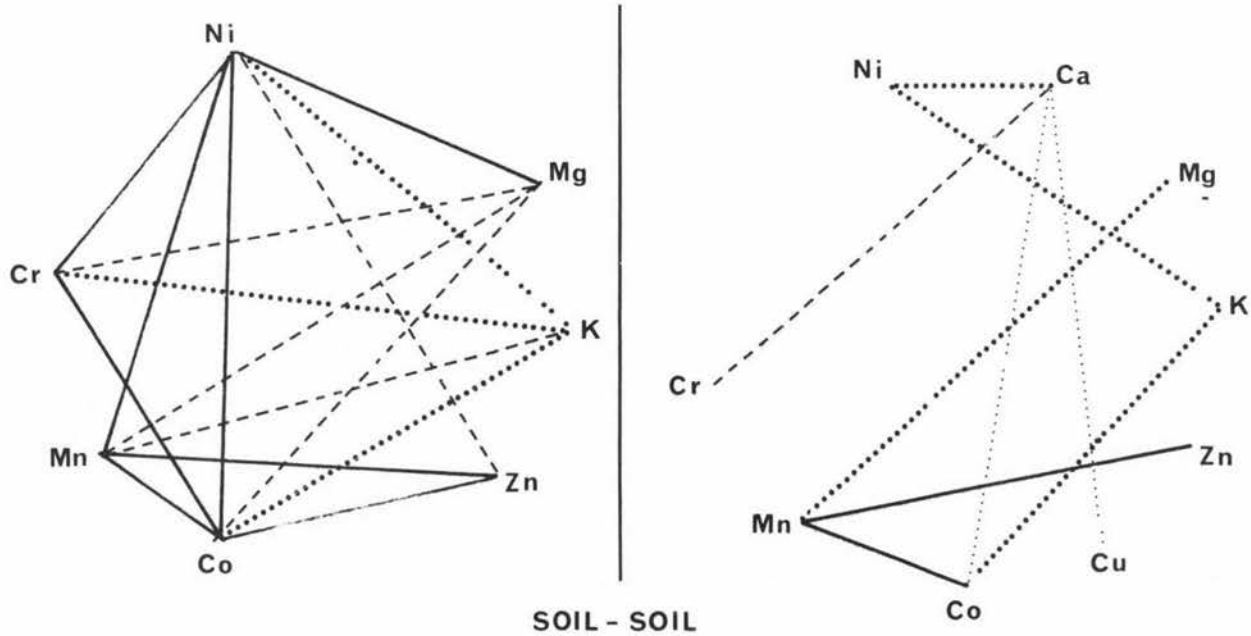
Fig. III-5a Element pla
and soil correlations.



PLANT - PLANT



PLANT - SOIL



SOIL - SOIL

H. odora

M. monroi

FIG.5b Element plant and soil correlations.

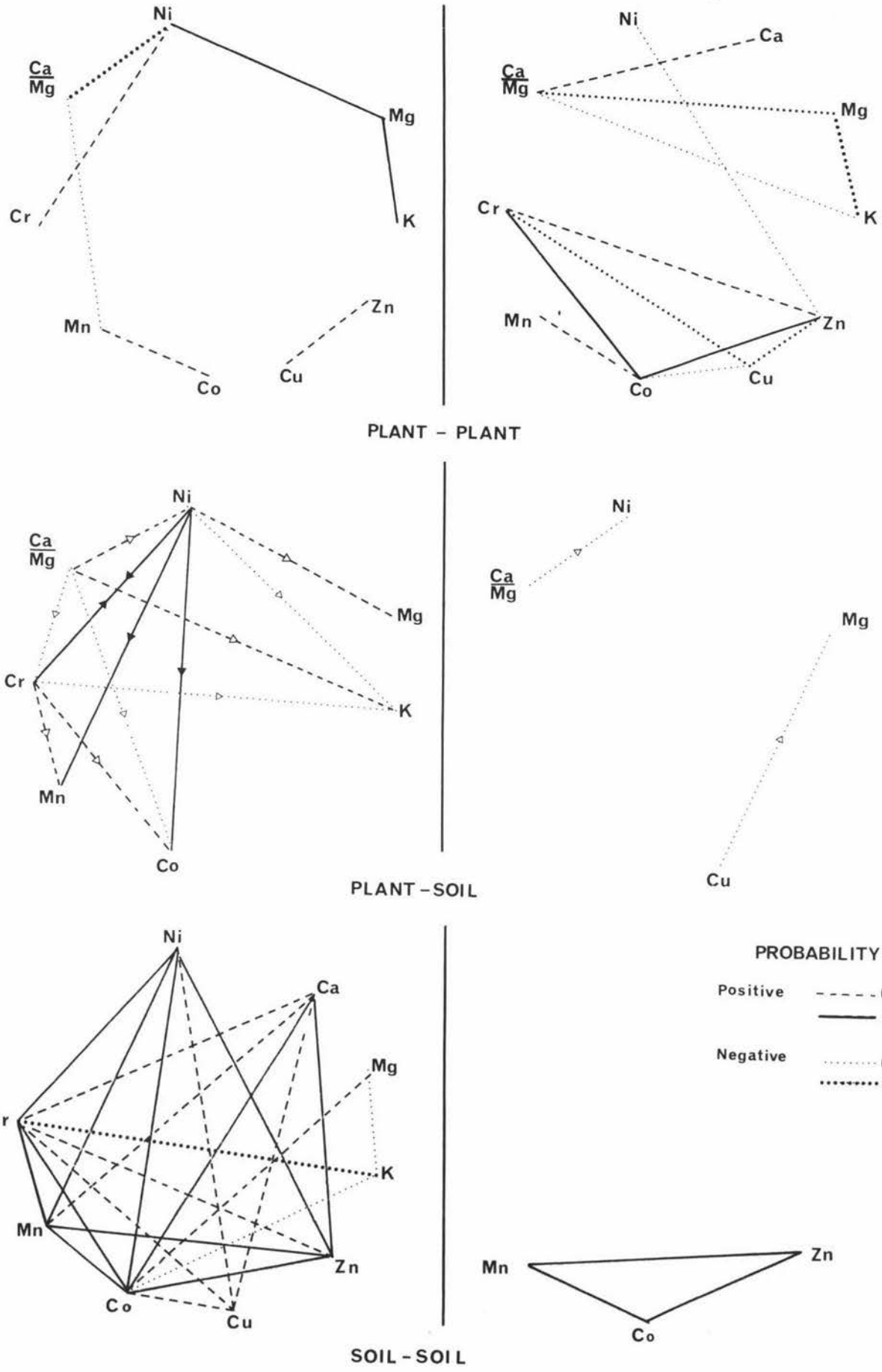


Fig. 5c Element plant and soil correlations.

SPECIES	Ni	Co	Cr	Zn
P. suteri (26)	+0.35 SIG 0.037	+0.44 SIG 0.011		
M. monroi (29)	+0.44 SIG 0.009			+0.35 SIG 0.030
H. odora (18)	+0.86 SIG 0.001	-0.64 SIG 0.002	+0.41 SIG 0.042	
C. vauvilliersii (19)				
L. Scoparium (23)	+0.75 SIG 0.001		+0.64 SIG 0.001	

TABLE III-5 Plant-Soil Correlations
(Only those where $P < 0.05$ are shown).

There was a strong antagonistic relationship between cobalt in the plant and in the soil for H. odora. Comparing this with the mean concentration of cobalt in Hebe-bearing soils (176 ppm) and the mean cobalt concentration in the plant ash (23 ppm) together with the small standard deviation of the element in the plant (see Fig III-4), it was seen that this plant must have a very efficient exclusion mechanism for cobalt and only accumulates what it requires for certain metabolic functions. (Cobalt is certainly toxic in not too large doses but nevertheless is an essential trace element).

C. vauvilliersii is the only species not showing a significant relationship between nickel in the plant and soil. The strongest correlation is shown by H. odora. This plant takes up nickel in amounts proportional to the soil concentration and its behaviour towards this element was quite different to that of cobalt. M. monroi showed a marginal correlation between zinc in the plant and soil, and was the only plant to do so. The standard deviation of zinc in the ashed leaves for this plant was lower than the others. As found by Lyon et al (1971) chromium in L. scoparium leaves and soils showed a high positive correlation ($P < 0.001$). The mean ashed content was also the highest of the five species studied. Lyon points out that this plant may be able to alleviate toxicity by being able to extract large amounts of calcium from the soils upon which it grows.

In general, strong correlations of the same elements between plants and soils is likely to be related to the wider variance of some elements over others.

However inter-element relations between the concentrations of elements in the plant and soil are more complex. Hebe odora showed a complex pattern of inter-relationships (fig III-5b) whilst the only correlations between the plant and soil of C. vauvilliersii are negative ones of copper in the plant and magnesium in the soil; and of the calcium/magnesium ratio in the plant and nickel in the soil (fig III-5c).

P. suteri showed a strong relationship of nickel, calcium and magnesium in the plant with potassium in the soil. This plant was found to

grow on very high potassium-enriched soils compared with the other four species (table III-2). H. odora and L. scoparium showed a negative nickel-potassium correlation. H. odora also showed a highly negative correlation between magnesium in its leaf ash and potassium in its soil. Of some interest was the only significant two-way correlations found amongst the species. These are shown in the illustration for the plant-soil relationships of H. odora. Nickel in the plant is negatively correlated with cobalt in the soil and vice-versa, whilst nickel was positively correlated with manganese in the soil which was also true the other way round, but at a higher probability level. L. scoparium showed a positive nickel, cobalt correlation between plant and soil.

2. Multivariate Analysis

The discriminant function is one of the most widely-used multivariate procedures used in earth science (Davis, 1973).

On the basis of several elemental concentrations discriminant analysis was used to distinguish the extent that the five different groups differed, and also to distinguish between the soils supporting endemic plants (E), and those supporing non-endemics (N). As preliminary investigations showed that the element concentrations are nearly lognormally distributed, the variables used were the logarithms of the element concentrations.

A set of linear functions

$$Z + L_1X_1 + L_2X_2 + \ldots\ldots\ldots L_kX_k + C$$

was calculated, in which X_1, X_2, $\ldots\ldots X_k$ are the logarithms of element concentrations, L_1, L_2, $\ldots\ldots$ K_k are weighted coefficients, and Z is the score of the discriminant function. The problem is now

reduced to finding the linear function which maximizes the differences in the score between the groups involved.

Discrimination was examined, (i) between the two groups E and N and (ii) among the five groups corresponding to the five sep rate plant species. On the basis of the best discriminant functions obtained, the plant expected to be found on each soil was predicted, and the number of correct assignments (i.e. the predicted species that was actually growing on the site) was tabulated. A Burroughs B6700 computor was used for the computations made.

(i) Discrimination between Endemic and Non-endemic species.

(a) Single-element discrimination.

Table III-6 shows the degree of ability of each of the elements studied to discriminate between the two groups. The table shows for each element the difference between the mean values of X (which is a logarithm) for the two groups ($\bar{X}_E - \bar{X}_N$), the concentration C giving maximum discrimination, and the number of correct assignments. Taking the best discriminating element, magnesium, as an example, the table shows that 52 of the 55 soils bearing endemic plants had magnesium concentrations above 12.20%, and 41 of the 60 supporing non-endemic plants had magnesium concentrations below this value. This gives a total of 93 correct assignments based on magnesium. It must be remembered that there is always a 50:50 chance of making a correct assignment to two equal groups, therefore at least 60 correct assignments can be made from the present samples, even with a completely

TABLE III-6

Endemic/non-endemic discrimination of a single-element basis.

Element	$\bar{X}_E - \bar{X}_N$	Conc.(ppm) for maximum discrim. (C)	Maximum degree of discrimination			
			No. of endemics	No. of non-endemics	Total correct	% of overall total
Mg	0.27	122,000	52 $\geq$ C	41 $\leq$ C	93	81
Ni	0.19	2,180	55 $\geq$ C	23 $\leq$ C	78	67
Mn	0.10	2,090	45 $\geq$ C	27 $\leq$ C	72	63
Co	0.10	193	52 $\geq$ C	17 $\leq$ C	69	60
Cr	0.09	1,290	10 $\geq$ C	56 $\leq$ C	66	58
Zn	-0.03	93	38 $\leq$ C	36 $\geq$ C	74	64
K	0.03	1,260	36 $\geq$ C	34 $\leq$ C	70	61
Cu	-0.01	75	19 $\leq$ C	47 $\geq$ C	66	57
Ca	-0.01	9,300	30 $\geq$ C	35 $\leq$ C	65	56

random distribution of element concentrations. No other element approaches the discriminating ability of magnesium, the next best being that of nickel. It can be seen that all of the endemic species grow on soils with nickel concentrations greater than C. That nickel is the second-best discriminator may be a reflection of the fact that nickel is the element whose concentration is most highly correlated to that of magnesium (correlation coefficient 0.82).

(b) Multi-element discrimination

Discriminant analysis was carried out with the Burroughs B6700 on a multi-element basis. As was expected from the results of the single-element discriminant functions, all of the most successful multi-element functions contained the variable magnesium. As can be seen from table III-7, very little improvement was achieved by including other variables. The table shows the number of correct assignments found with the best linear combinations from selected groups of elements. The dominance of magnesium is further shown in the magnitudes of the weighting coefficients L_i after normalization of the variables.

(ii) Discrimination among the five species

Discriminant analysis among the five soil groups corresponding to a particular plant species, was investigated to show whether certain species had specific preferences for certain elements. It was seen from table III-2 that the two endemic species, both predominantly growing in high-magnesium soils, favoured areas of quite different potassium and copper concentrations. P. suteri favoured high potassium/low copper soils whilst M. monroi prefered low potassium/high copper soils.

The results of various element combinations and their degree of discrimination among the five species are shown in table III-8. Only the most successful of the single -, two -, and three - element discriminators are shown. Combinations of four or more elements did not add to the discrimination shown by the Mg-K-Cu combination.

TABLE III-7

Endemic/non-endemic discrimination on a multielement basis.

Element combination	No. of correct assignments	Percentage of total
Ni	77	67
Mg	91	79
Ni-Mn	80	70
Mg-Ni	91	79
Mg-Mn	93	81
Mg-K	95	83
Ni-Mn-Zn	84	73
Mg-Ni-Cr	91	79
Mg-Ni-Zn	91	79
Mg-K-Cu	95	83
Mg-K-Mn	95	83
Mg-K-Ni	96	84
Mg-K-Ca	96	84
Mg-K-Mn-Ni	96	84
Mg-K-Cr-Ni	96	84
Mg-K-Ni-Ca	97	84
Mg-K-Mn-Ni-Zn	97	84
Mg-K-Ni-Ca-Cr	97	84
Mg-K-Ni-Cr-Mn	98	85
Mg-K-Mn-Ca-Cr	98	85

TABLE III-8

Discrimination amoung five plant species

Table shows number of correct assignments in each group and total number of correct assignments

Element(s)	P. suteri	M. monroi	H. odora	C. vauvilliersii	L. scoparium	Total
Zn	14	5	13	4	2	38
Ni	12	18	4	3	2	40
Cu	19	15	5	4	2	45
K	19	15	1	4	6	45
Mg	12	21	6	7	2	48
Cu, Mn	18	14	3	1	10	46
K, Mn	23	11	0	6	6	46
K, Ni	19	15	3	13	5	55
K, Mg	24	22	5	13	5	69
Cu, Ni, Mn	18	20	5	5	11	59
Mg, Cu, Ca	20	25	7	1	7	60
Mg, K, Ca	24	23	5	13	6	71
Mg, K, Cu	25	23	6	11	9	74
Total samples in each group	26	29	18	19	23	max. 115

The high score of this combination results largely from the tendency of the endemics and non-endemics to discriminate between high and low magnesium sites and the preference for potassium and copper levels at a specific level. The K-Ni and K-Mg combinations result in a reasonable score for C. vauvilliersii var serpentina (13 out of 19). This may be expected as the potassium levels are generally lower for this species substrate than for the other two non-endemics (see fig III-2). Apart from this, there is no element or combination that discriminates strongly among the three non-endemic species.

D. DISCUSSION

1. The influence of high magnesium in the soil

The most obvious trend to emerge from the above work is the marked influence of magnesium in controlling the distribution of endemic and non-endemic species. This may result simply from a tolerance by the endemic species to high magnesium concentrations. It does not necessarily mean that magnesium itself is directly responsible for the predominance of P. suteri and M. monroi in such localities. It is known that high levels of magnesium are associated with low extractability of calcium (Walker, 1954 and Lyon, 1968). Thus the endemic plants may be able to survive where the already low calcium levels are further decreased by the high levels of readily-extractable magnesium. In areas more favourable to other plants the endemics may be unable to compete. Proctor (1970) provided support for the importance of high magnesium. Others have emphasized the importance of low calcium, e.g. Kruckeberg (1954),

Proctor (1971) investigated the growth of oats on three serpentine soils with varying degrees of magnesium concentrations and deduced from plant analysis, and the symptoms shown by the oats when growing in these soils, that high levels of magnesium in the presence of low calcium levels were responsible for the poor growth of the oats. In the serpentine soil where the magnesium excess was not as great, toxicity symptoms were correspondingly less severe. Proctor (1971) also demonstrated races of oats that were resistant to magnesium toxicity in water cultures and correlated this with their rooting in high magnesium soils. A debris soil from Rhum which was very impoverished in plant nutrients, did not appear to be toxic to the plants investigated, and a species of Agrostis collected from this site was not tolerant to magnesium and did not produce roots in the very high magnesium containing soils. He concluded that high magnesium/calcium ratios are likely to be of widespread though not of universal importance in serpentine ecology. The Meikle Kilrannoch soil, which had the highest magnesium/calcium ratio represented an extreme in toxicity whereas others with high magnesium/calcium ratios caused a hinderance only. The data Proctor obtained suggested that ecotype adaptation to magnesium toxicity is widespread in British and Swedish serpentine soils.

At this stage the results of the soil survey at Dun Mountain seem to agree with these findings. The endemics which are the best adapted to high magnesium levels are restricted to such areas where competition from the less magnesium tolerant species is minimized. The serpentine ecotype are

in turn, able to withstand higher magnesium concentrations than the non-serpentine species.

Ferreira (1964), from his experimental data, concluded that 'high magnesium content is just as toxic to calciphiles as heavy metal content of the soil'.

Proctor (1971) has shown that toxicity depends not only on the relative amounts of calcium and magnesium present but that it is also a function of the concentration of these elements.

It was obvious that plant species varied in their tolerance to magnesium and to a low calcium/magnesium ratio. This was borne out by the large differences in the mean values for magnesium in the plant species studied, and also in the soils upon which they grew on. The magnesium concentration in the dried leaves of the two endemic species was approximately 4 times that of the non-endemics. The calcium concentrations did not follow such a clear-cut pattern. In fact the dried leaves of M. monroi, an endemic, have the highest calcium content even though it is a non-woody plant. For the non-endemics, the calcium/magnesium ratio was nearly always above 1. Walker et al (1955) showed that different magnesium concentrations had a large effect on vegetation and that this soil factor was more important for certain species and may vary from site to site. Calcium concentrations on all sites were fairly constant although this does not necessarily reflect the exchangeable calcium pattern.

Ritter-Studnicka (1972) showed that some species appeared to be able to control the uptake of calcium and magnesium from the soil

whilst others absorbed magnesium and calcium randomly. Thus the ability of individual species to grow on serpentine soils was a result of their physiological constitution. In this study there were no strong correlations between calcium and magnesium in the plant with those in the soil. This indicated a random uptake. There were however prefered sites; the endemics preferring, or more likely restricted to, high magnesium areas which were above some threshold level for the other serpentine growing species. Note the low standard deviation for magnesium in endemic soils and the wide spread of values for the non-endemics. Using the dry weight mean concentrations for magnesium we find that the relative accumulations (plant concentration divided by that in the soil) for the two endemic species were twice as high as the non-endemics. Thus as well as being able to grow on higher magnesium soils the plant makes no attempt to restrict uptake but seems to prefer to concentrate the element. The findings therefore seem to point to a physiological difference between the endemics and non-endemics as regards their behaviour towards magnesium. The non-endemics were ; adapted to the high concentrations, but within certain limits, whilst the endemics 'thrive' on high magnesium levels and perhaps have a physiological need for it. Pot trials seem to be indicated here to observe the growth of endemics on low magnesium soils. The non-endemics are certainly not completely at home in serpentine soils as observed by stunting and other morphological symptoms, compared with species growing in other areas, but have perhaps adapted 'ecotypes' due to their very ubiquitous nature. (N.B. To date

there is no evidence that the non-endemics are in fact ecotypes).

Relative accumulation of calcium by the five species showed no distinction between the endemics and the non-endemics: L. scoparium and M. monroi having the highest calcium-accumulating ability. (Calculated on a dry weight basis. It is important when making such comparisons that data be converted to a dry weight basis as, due to differences in organic content, certain false anomalies may occur. This is evident with M. monroi which seemed to have a very low organic content compared to the other four species. Note that the percent ash was roughly five times that of the others (Table III-4). Comparing the magnesium content in the ash of P. suteri and M. monroi there was a very large difference but both plants showed nearly the same concentration on a dry weight basis).

2. The influence of nickel, chromium and cobalt on serpentine flora.

It is recognised that the uptake of heavy metals depends on their solubility status in the soil; this having a significant bearing on the form in which they exist. For instance do nickel and chromium exist as Ni^{2+} and Cr^{3+} respectively or do they occur predominantly as inorganic or organic complexes which may be completely innocuous to plant growth? From a biological standpoint therefore, only the plant-available part of the element in the soil is effective on plant growth. As pointed out by Ernst (1972) the lowest plant-available level is that which is water-soluble with the exchangeable amount constituting the highest level.

These amounts are determined by pH, organic content and binding capacities of the clay constituents. Ernst (1968) showed that essentially organic metal compounds are taken up by plants to a lesser degree than inorganic ones. Ernst (1972) gave total, ammonium acetate-exchangeable and water-soluble nickel values from a South-Central African heavy metal soil as 4800, 100 and 0.2 ppm. Values for a typical soil from Dun Mountain were 3,000, 15 and 1.5 ppm. It is evident that the exchangeable portion of nickel is considerably less in these soils. There was an even greater difference between exchangeable chromium values (30 versus 2.5 from similar total chromium concentrations). These values point to the different behaviour of nickel and chromium in the two soils and has important significance for their possible toxic qualities. Exchangeable nickel is not greatly higher than the toxicity level (see Section III-B-2) and it is maintained by the author that the role of nickel as a toxic element in predicting the distribution of species in the Dun Mountain serpentine area is not as great as that of magnesium. There may well be of course species not present at all in the area which may be susceptible to the existing nickel presence. Previously it has been established that chromium exists predominantly in an inaccessable form for plants, although that which is available, is taken up to varying degrees by different species. Proctor (1971) has shown that nickel and chromium are very toxic and that in the absence of their cations, extremely low levels arrest the growth of roots of Agrostis spp. However various soil conditions may modify this toxicity and

completely innocuous comflexes may occur. Other elements also affect the degree of toxicity symptoms. Crooke and Inkson (1955) showed that when calcium and potassium had low concentrations, nickel toxicity was greatest. Note that the potassium level in P. suteri soils was very high and so was the significance of . plant-soil correlations between nickel, calcium and magnesium in the plant with potassium in the soil (fig III-5a). Thus, although the two endemics are unable to prevent nickel accumulation, the presence of relatively high-calcium and potassium in the leaves may alleviate any toxic effects. On a dry weight basis, these two plants have higher potassium levels in their leaves than the non-endemics and also relatively high calcium concentrations. (see Table III-4). There is evidence that chromium tends to remain in the roots and this will be seen to be true later.

Proctor (1971) raises the question as to "whether tolerance to nickel is merely linked with non-specific magnesium tolerance" as both nickel and magnesium toxicity may be ameliorated by calcium. However he cites a case where a particular species of Agrostis was tolerant to nickel but poisoned by magnesium. This seemed to be true of certain species on the "Mineral-Belt". Thus the possibility exists that heavy metals may only rarely be directly toxic but may have significant influence on other ions.

Proctor, concluded that nickel has some effect on some sites investigated (from different serpentine areas) but is not one of 'acute toxicity'.

Are toxic effects of heavy metals associated with high concentrations of the element in leaf tissue? This does not seem to be necessarily so.

M. monroi has high levels of nickel, chromium, cobalt and copper in its leaves but appears to be quite tolerant to these amounts. P. suteri also has high levels and it would be interesting to observe its growth behaviour on more "favourable" soils. Hunter and Vergnano (1953) found that the order of elements (Ni > Cu > Co > Cr > Zn > Mo > Mn) in producing chlorosis in comparable to the order of stability of metal complexes and suggested one factor in nickel toxicity may be the exhibition of one or more functions of copper. It was found that nickel was very highly correlated with copper in the leaves of P. suteri.

Hunter and Vergnano (1953) also showed that to consider chromium as a toxicity factor, the level of available chromium should be higher than that of available nickel. This is certainly not the case in these soils.

3. General conclusions

In summation the following characteristics may be noted.

a. Soil characteristics

(i) The generally high levels of magnesium in the serpentine soils with the soils supporting endemic plants having approximately twice the levels of those supporting non-endemics.

(ii) The low, but constant levels, of total calcium in all the soils sampled.

(iii) Low level of potassium but much higher in soils from P. suteri sites.

(iv) The high soil levels of chromium, nickel and cobalt with levels only marginally higher in endemic-supporting soils.

(v) Low level of available chromium. Also supported by Menezes de Sequeira (1968).

(vi) Higher level of available nickel usually exceeding toxicity levels. Observed in other serpentine soils also (Hunter and Vergnano, 1952; Hunter, 1954; Spence, 1957).

(vii) Soil pH fairly constant at 6.5. Similar to those reported by Lyon (1969).

(viii) Low calcium/magnesium ratio. Also a general characteristic of other serpentine soils; Proctor (1971), Whittaker (1954), Walker et al (1955), Soane et al (1959), Crooke (1956).

(ix) The large number of highly-significant elemental correlations in the soils of the five groups but that between manganese and cobalt is the only one common to all.

(x) Magnesium is far superior to the other elements in giving maximum discrimination between soil groups for endemics and non-endemics.

b. Plant characteristics.

(i) High magnesium values in the leaves of the endemics.

(ii) High nickel concentrations in the leaves of the endemics. Approximately 10 times those in the non-endemics (refer dry weight table).

(iii) Calcium/magnesium ratio below a value of 1 for the endemics but greater than 1 for the non-endemic species.

(iv) The higher relative accumulation of magnesium and nickel for the endemics.

(v) The general differences in variation of elements between non-endemics and endemics.

(vi) Characteristic vegetation is due to a true "serpentinic effect" and not adverse physical or climatic effects.

Comparing the New Zealand serpentine area at Dun Mountain with the ultramafic complex in New Caledonia, there are two major differences that are notable when looking at the respective soil element concentrations. First there is the lower nutrient content (Brooks et al 1973) of the New Caledonian soils; a difference in the order of 100. Thus New Caledonian soils appear to be even more unfavourable for plant growth yet these soils support a more "luxuriant" vegetation. (Jaffré, et al, 1971) than the Dun Mountain serpentine soils. Admittingly the climates and soil conditions differ greatly. Secondly, however is the difference in magnesium levels, those at Dun Mountain being considerably higher and comparable with those of Aberdeenshire serpentines (Proctor, 1971). The chromium content of some New Caledonian soils is of the order 2-3 percent whereas it is noticed the Dun Mountain soils are always less than 0.5 percent, again these compare favourably with values for Aberdeen serpentine soils.

It therefore seems that the high magnesium levels in the Dun Mountain soils are suppressing calcium and the nutrient uptake by the plants. (Calcium/magnesium ratios are similar in both soils). Magnesium, calcium and potassium would be readily leached from the tropical laterites of New Caledonia but the insoluble chromite would accumulate in the soil profile. Thus the

factors operating in Dun Mountain serpentines, and seemingly supported by Proctor (1970, 1971), are quite likely to be different to those in New Caledonean ultramafics. Some plants on these soils are observed to accumulate tremendous amounts of nickel (Jaffré et al, 1971). The next section will deal with the plant chemistry of one of these plants, Hanalium kanaliense.

SECTION IV

PLANT BIOCHEMISTRY OF NICKEL

A. INTRODUCTION

The work in part III shows that some plants are able to accumulate substantial quantities of nickel particularily *P. suteri*. However, recently, Jaffré *et al* (1971) and Jaffré (1973) have reported *Hybanthus* species and others that contain extraordinarily high levels of nickel in their leaves. Quantities of one and two percent of dry leaves being not uncommon. Jaffré and Schmid, (1974) have reported yet another accumulator of nickel: *Psychotria douarrei*, which contains amounts up to 4.5 percent (40 + percent in the plant ash). This level is by far the highest reported for any element, in any species, and such a level must surely have outstanding physiological implications. Brooks *et al* (1973) have listed some nickel accumulating plants and reported new data for the New Caledonian species, *Homalium kanaliense* and the two violaceae, *Hybanthus austro-caledonicus* and *H. caledonicus*.

It seems that accumulating ability is often a common factor among several species of one genus. For example the Hybanthus members, *H. austro-caledonicus* and *H. caledonicus* (Brooks *et al*, 1974) from New Caledonia and *H. floribundus* (Severne *et al*, 1972) from Western Australia have the ability to accumulate very large amounts of nickel. This feature is also found in *Alyssum* species (Vergnano, 1958; Malyuga, 1964, and Menezes de Sequeira, 1968). Two *Homalium* species from New Caledonia have been reported, and also contain large quantities of nickel; *H. kanaliense* (Brooks *et al*, 1974) and *H. guillainii* (Jaffré, 1974). *H. kanaliense* is investigated further in this present study.

In the literature can be found work on other trace metals. Chromium in Leptospermum (Lyon, 1969), copper in Becium homblai (Reilly, 1967, 1969), uranium in Uncinia (Whitehead, 1970) and zinc, copper and lead in ryegrass, Agrostis tenuis (Bradshaw, 1969; Peterson, 1967) and recently preliminary observations on the plant chemistry of nickel in some of the New Caledonian accumulators (Kelly, et al, 1974). Tiffin (1971) has investigated the translocation of nickel in the xylem exudate of plants and also the transport of manganese, iron, cobalt and zinc in tomato plants (1967). Tiffin (1966) provides evidence for the translocation of iron as an iron-citrate complex. Bremner and Knight (1970) have studied the complexes of zinc, copper and manganese in ryegrass, but although obtaining knowledge on their behaviour, they have not given any exact identification on their forms. This has been the major trend so far and very little is known on the actual forms in which trace elements occur in plants.

Plant chemistry studies were carried out mainly on Homalium kanaliense with some work on P. suteri and P. douarrei in the hope of advancing the present knowledge of nickel in the plant system and the ultimate goal of establishing its exact identity within the species H. kanaliense. This plant was chosen because of its availability and its high nickel content. Some preliminary work was carried out on P. suteri.

For a plant to accumulate great quantities of a trace element, protective mechanisms may be necessary and of these the most attractive is chelation, as this effectively removes the

cation in question from possible general reactions. It prevents the cation from inhibiting certain essential reactions. There is no sound evidence to date of nickel having an essential role and it is better known for its highly toxic qualities, yet large amounts are still taken up by certain plants. The most important toxic action is thought to be the poisoning of enzymes (Bowen, 1966). Chelation by various organic substances present in the plant may prevent such action, as chelates have very high stabilities, and many of the cations biological, chemical, and physical properties are changed. Poole and Poel (1965) say, that, in general cation uptake is often balanced by organic acid synthesis. Ritter-Studnicha (1972) noticed this also. They observed that total acids were higher in serpentine plants than those of calcium-rich soils, and the production of organic acids appeared to be stimulated by magnesium accumulation. This appears to be in good agreement with the facts found so far on serpentine plants, particularly the endemics, where uptake of nickel is very highly correlated with magnesium concentration in the plant.

Not very much information is available about naturally-occurring chelating substances within plant tissues but it appears that metals are frequently translocated as chelates (Timberlake, 1959; Tiffin 1966, 1967, 1971). E.D.T.A. is extremely effective in alleviating toxicity from heavy metals as it forms very stable chelates (Chenowith, 1956).

A brief look at the chemistry of nickel and possible chelating agents is perhaps required at this moment.

Unlike some other transition elements, nickel commonly shows only the + 2 oxidation state in aqueous solution. The hexaquo ion, $Ni(H_2O)_6^{2+}$ is quite labile and barely acidic (Cotton and Wilkinson 1962). In complex compounds Ni (II) is rather peculiar in that stereochemical transformations occur fairly easily. This is often described as the "anomalous" behaviour of Ni(II) complexes. Six-co-ordinate (II) complexes are almost always high-spin complexes having either regular or distorted octahedral chemistries. The ground state term (^3F) is split in an octahedral field giving rise to the triplet terms with three spin-allowed transitions. These three transitions are observed in regions, 7000-13000 cm^{-1}, 11,000 - 20,000 cm^{-1} and 20,000 - 28,000 cm^{-31}. Nicholls (1973) gives a excellent review of the general chemistry of nickel.

There are many groups of compounds occurring in plants from which nickel complexes may arise e.g. amino acids, organic acids, phenolic compounds, proteins etc. The amino acids in particular have a strong ability towards chelation of nickel (II). There are some compounds in plants which do not possess suitable functional groups, notable ones being the lipids and the carbohydrates.

Many nickel (II) chelates are quite simply made. The use of potassium oxalate in the formation of oxalato nickel complexes leads to salts such as $K_2Ni(C_2O_4)_2\ 6H_2O$ (Martell and Galvin, 1952). Hughes (1972) also points out that nickel inhibits enzymes and in some cases enzymes are known which are activated by nickel.

TABLE IV-1

Stability Constants for some Nickel complexes

Chelating Ligand.	Constant	log K*	References
Glycine	K_1	5.77	Gillen and Martell (1964)
	K_2	4.80	" " "
Aspartic Acid	K_1	7.12	" " "
	K_2	5.27	" " "
Histidine	K_1K_2	15.90	" " "
Glutamic Acid	K_1	5.90	" " "
	K_2	4.44	" " "
Lysine	K_1K_2	8.8	" " "
Ammonia	K_1	2.79	" " "
	K_2	2.24	" " "
	K_3	1.73	" " "
Acetate	K_1	0.67	" " "
E.D.T.A.	K_1	18.56	" " "
Proline	K_1K_2	11.3	Martell and Galvin (1952)
Citric Acid	K_1	5.11	Li et al (1959)
	K_2	3.19	" " " "
Salicylaldehyde	K_1	5.22	Martell and Galvin (1952)
	K_1K_2	9.2	" " "
8. Hydroxyquinoline	K_1	11.65	" " "
	K_1K_2	22.0	" " "
Oxalic Acid	K_1K_2	6.51	" " "
Malonic Acid	K_1	4.00	" " "

* log K values are the log equilibrium formation constants.

Table IV-1 lists some selected nickel complexes and gives an idea as to their stability. Note the high stability of the E.D.T.A. nickel complex, already mentioned and the powerful chelating agent, 8-hydroxyquinoline. Rubin et al (cited from Martell and Calvin, 1952) injected many times the toxic dose of nickel into humans, as the E.D.T.A. complex and no adverse symptoms were observed. The nickel, as the chelate, was eliminated in a few hours. As seen from the table, histidine also complexes strongly with nickel.

Thus, in the plant cell there are a number of different species which are potentially able to chelate with nickel and in so doing alleviate some of its toxic properties. The research in this section was carried out in an effort to establish the character of nickel within the plant since it was deduced that some sort of chelation may take place. A further aim was to establish the proportion chelated, and to see if there are any specific binding localities in the plant leaf. A preliminary investigation established the distribution of nickel in the carious plant organs. The New Caledonian species, H. kanaliense was used in the majority of the plant chemical work, and the other nickel accumulators P. suteri from Dun Mountain and Psychotria douarrei from New Caledonia were used in some cases for comparison purposes.

B. INTRAPLANT DISTRIBUTION OF NICKEL AND OTHER METALS.

1. Samples and Methods

Specimens of Pimelea suteri were collected from sites on the Dun Mountain ultramafic belt described in Section I. Leaf material from several plants was carefully washed and subjected

to freeze-drying. The material was then ground to a fine mesh size and stored until use, in a sealed container under refrigerated conditions. Several other speciments were dissected into their various parts, ashed, and the nickel and other elements were analysed by atomic absorption.

Leaf material from the New Caledonian species, H. kanaliense and P. douarrii was also freeze-dried and ground. This material was used in various solvent extractions from which fractions were obtained for further study. These and other New Caledonian species which accumulate nickel are described by Jaffré et al (1971), Brooks et al (1974) and Jaffré (1973).

The three species named above were all used in amino acid studies while freeze-dried material from H. kanaliense was used for plant chemical studies of nickel complexes.

2. Distribution of nickel and other elements in various plant organs.

Specimens of P. suteri were divided into the various components given in table IV-2. The various plant organs were ashed and analysed by atomic absorption as in section II. The results are shown in table IV-2.

It is interesting to note the high concentrations of nickel in the outer bark of both the stems and the roots. The inner stem tissue contains the least nickel. Similar trends were observed for chromium, thus is appeared that nickel and chromium uptake was somewhat restricted but not effectively. These results can be compared to those of other nickel accumulators shown in table IV-3. H. kanaliense contains about seven

times more nickel in the leaves than the bark. Chromium in both P. suteri and the New Caledonian species was restricted at the roots to a greater extent. This restriction of chromium at the roots has been observed by Hunter and Vergnano, 1953, who also stated that chromium is especially toxic to root development. P. suteri was observed to have very long tap roots for such a small plant: 35 inches in one case, but little development of fibrous roots. A large difference between the calcium/magnesium ratios of the New Caledonian species and P. suteri was noticed: those for P. suteri being much lower, although the calcium concentrations are higher in Dun Mountain soils. It seemed P. suteri was a much greater accumulator of magnesium but this was probably because of the much higher magnesium concentrations in the soil. It was also noticed that levels of the nutrients, calcium, potassium and phosporous, are higher in the New Caledonian plants than in P. suteri.

Correlation analyses on 26 samples of leaves of P. suteri showed the following highly significant correlations ($P \leq 0.001$) between element pairs : nickel-cobalt, $r = 0.65$; nickel-copper, $r = 0.59$; nickel-zinc, $r = 0.61$ and nickel-magnesium, $r = 0.73$. There were also highly significant correlations between chromium and cobalt, $r = 0.76$; chromium copper, $r = 0.64$ and cobalt and copper, $r = 0.64$. There were no significant inverse relationships. Other correlations at the $P \leq 0.01$ level are shown in fig III-5a.

P. suteri	% Ash	ppm							%		
		Ni	Co	Cr	Cu	Zn	Mn	Pb	Fe	Ca	Mg
Leaves	5.35	2576	34	692	173	761	2422	173	2.77	8.99	22.14
Twigs (Young)	2.65	2210	63	136	187	1802	1292	238	1.70	9.52	10.2
Stem (peeled)	0.97	1680	N.D	252	215	1743	1050	210	1.26	6.03	9.88
Outer bark	3.83	5340	320	1281	160	925	2598	106	6.40	3.20	12.82
Inner bark	2.20	2257	N.D.	387	258	1677	1419	258	1.93	9.67	9.03
Main root	4.95	2500	112	1250	100	812	1375	13	4.75	5.00	9.50
Fibrous root	6.87	4200	120	1824	144	576	1776	72	6.72	6.24	11.52
Peeled root	2.20	1242	N.D.	552	138	1794	1049	138	1.10	7.17	6.62
Root peelings	4.90	5137	164	1150	205	1356	2055	102	6.16	6.57	8.22
Soil	86.33	4000	250	2600	440	155	2400	30	23.00	0.50	15.00

TABLE IV-2 Elemental concentrations (Ash wt) in various organs of several representative P. suteri plants. (Five specimens combined).

TABLE IV-3

Elemental concentrations (% ash weight) in various organs of representative individual nickel-accumulating plants and their soils.

	Co	Cr	K	Ni	P	Ca	Mg	Ca/Mg
Alyssum bertolonii *								
Seeds	-	-	-	7.20	-	17.30	4.60	3.96
Flowers	0.030	0.000	-	6.40	-	20.30	4.50	4.50
Mature leaves	0.001	0.019		8.00	-	23.30	4.60	5.06
Roots	-	-	-	4.60	-	13.70	6.00	2.28
Soil	-	0.560	-	0.26	-	-	-	-
Homalium kanaliense								
Flowers	0.020	0.006	39.75	2.93	1.12	13.05	6.00	2.18
Mature leaves	0.042	0.012	36.00	7.48	0.56	13.95	3.60	3.87
Bark	0.006	0.006	6.45	1.01	0.15	3.45	0.90	3.83
Soil	0.054	2.964	0.005	0.49	0.003	0.001	0.10	0.01
Hybanthus austro-caledonicus **								
Flowers	0.009	0.016	46.50	0.90	3.75	8.40	12.90	0.65
Old leaves	0.048	0.079	17.25	24.00	0.84	10.50	13.80	0.76
Mature leaves	0.027	0.036	19.90	22.50	1.14	9.60	14.25	0.67
Twigs (2-year)	0.004	0.014	16.20	6.75	0.53	7.65	3.00	2.55
Bark	0.016	0.016	-	21.00	0.51	6.90	2.25	3.06
Thick roots	0.004	0.028	5.40	6.67	0.32	6.75	2.10	3.21
Fine roots	0.008	0.108	5.40	18.00	0.51	2.70	3.45	0.78
Soil	0.062	2.128	0.005	0.80	0.003	0.08	1.68	0.05
Pimelea suteri								
Mature leaves	0.009	0.015	14.00	0.59	0.60	7.0	28.00	0.25
Twigs	0.025	0.034	12.00	0.75	0.88	5.4	24.00	0.22
Roots	0.015	0.067	1.86	0.53	5.26	3.5	17.50	0.20
Soil	0.040	0.160	0.32	0.35	0.04	0.5	15.00	0.03

*Data from Minguzzi and Vergnano (1948) and Vergnano (1958).

** Brooks *et al* (1973).

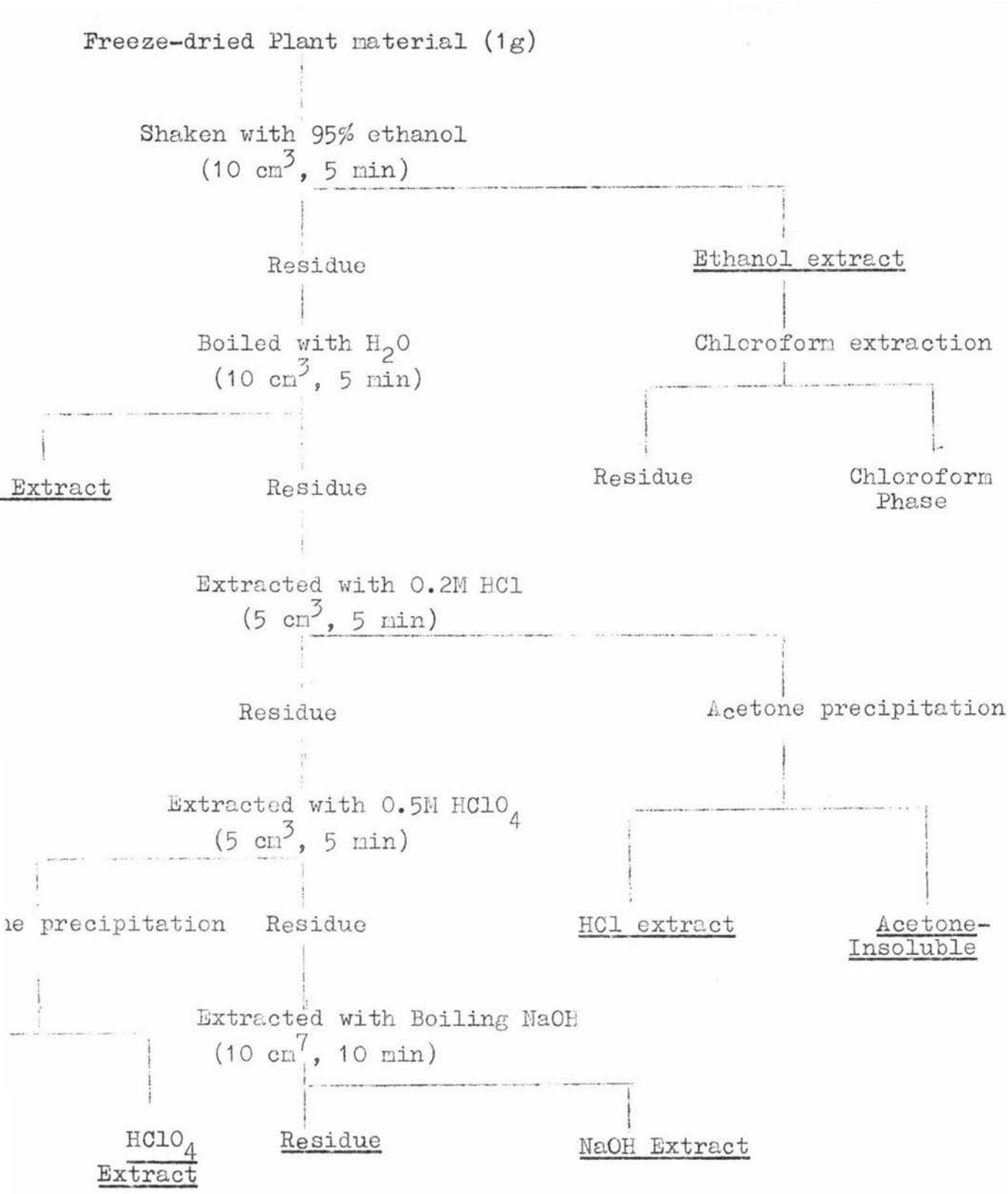


FIG IV-1 Solvent Extraction Scheme
After Bowen et al (1962)

3. Preliminary fractionation of plant tissue

Solvent extraction and differential centrifugation was carried out on freeze-dried material and fresh leaves of H. kanaliense to establish in what fractions nickel is most easily extracted into, and its whereabouts within the plant cell. The freeze-dried powder contained approximately 6,000 p.p.m. nickel. Some work was also done on freeze-dried P. suteri leaves which contained about 200 p.p.m. nickel.

(i) Solvent extraction

(a) Methods

Solvent fractionation methods similar to those of Bowen et al (1962) were followed. Eight fractions were obtained. The general scheme is shown in fig IV-1. Each fraction was reduced to dryness over a hot water bath in polypropylene beakers and the organics decomposed using 20 cm^3 of concentrated nitric acid. (Nitric acid was added to those samples containing perchloric acid before taking to dryness in order to oxidise most of the organic material before it could react explosively with perchloric acid. Acetone also reacts with hot nitric acid. This was therefore evaporated first). One gram of freeze-dried material was used and analyses were carried out in triplicate.

One gram of material was shaken for 1 hour in distilled water (20 cm^3) at 4^oC to establish the water-soluble quantities of nickel. The 50 cm^3 centrifuge tubes used were centrifuged at 5,000 r.p.m. and the supernatant liquid filtered to ensure all residues were removed. The aqueous liquid was analysed for nickel by atomic absorption spectroscopy.

Fresh-leaf tissue (5g) was homogenized for 60 secs, in 10 sec bursts to prevent temperature rise, in 100 cm^3 of distilled water at approximately 0^oC. This was achieved using a Waring blender at top speed. The supernatant was centrifuged and filtered.

Lipids and pigments in the aqueous phase were extracted using chloroform and this fraction also analysed for nickel.

(b) Results and discussion

The results of the solvent fractionation and the cold water extraction are given in tables IV-4 and IV-5. All data are the means of three replicates which generally agreed to within ± 20 percent. A chloroform extraction of the aqueous phase removed the majority of the lipids and plant pigments leaving a darker aqueous phase. The negligible amount of nickel extracted into the chloroform (0.01%) was probably due to contamination from the aqueous phase. Thus any association of nickel with pigments, lipids and other chloroform-soluble components could be ruled out. Lipids were concentrated at the interface of the two phases.

As seen from table IV-5, over 50 percent of the nickel was water-extractable. Note the much higher extractability of fresh leaves. This high water-solubility of nickel from H. kanaliense indicated that a large proportion at least existed as highly polar complexes; whether it was the hexaquo ion or some other complex remained to be investigated.

From table IV-4 it was seen that the trends for the four species were similar although P. suteri

tends to contain larger amounts in the soda fraction and the residue. Hot water and 0.2 M hydrochloric acid extracted more than 80% of the total amount of nickel from the three New Caledonian species and 60% from P. suteri. This indicated the high solubility, polarity and exchangeability of the nickel compound. Only minimal amounts were extracted by 95 percent ethanol. This fraction normally contains low molecular weight species of some polarity e.g. amino acids and some organic acids, and non-polar pigments and lipids. This suggested that the nickel is not attached to easily removed non-polar material and some kind of chelation probably existed.

The acetone-insoluble fraction contained most of the pectates and proteins. The absence of any appreciable quantities of nickel in these fractions indicated that it was not co-ordinated or associated in any way with any of these classes of compounds.

The perchloric acid fraction removed the remainder of the polar compounds and other more tightly bound groups remained in the residue, e.g. structural groups, such as cellulose and lignin. The soda fraction contained degraded proteins and polysacchorides. In the case of H. kanaliense some nickel remained in the insoluble residue suggesting stronger binding, however for the other two New Caledonian species practically all nickel was removed easily by the water extraction and the two acid extractions. Ernst (1970) showed that 23 percent of the nickel in Indigopera setiflora was water soluble, 34 percent soluble in citric acid and a similar amount in hydrochloric acid. Butanol extracted

only minimal amounts which were comparable to the ethanol fraction in this study. Ernst's work showed that generally leaves with high metal contents also have a considerable water extractable fraction and that metals extractable by water are more or less located in the vacuole system.

The amount of nickel found in the residue represents nickel immobile within the cell and may be bound to the cell wall. Comparing the four species in table IV-5 it was seen that binding capacity of nickel was the strongest in P. suteri (although this had a much smaller total amount). There was some binding of nickel in H. kanaliense, and in H. austro-caledonicus and P. douarrei practically all the nickel was water soluble or readily exchangeable.

TABLE IV-4

Percentage distribution of Nickel in leaf extracts

Species	Fraction							
	Ethanol	H_2O	HCl	Acetone insoluble	$HClO_4$	Nucleic Acid	NaOH	Residue
H. kanaliense	0.65	55.92	26.82	0.30	6.26	0.07	3.84	8.04
P. suteri	1.50	40.00	10.00	2.00	10.00	1.0	23.0	10.50
Hybanthus austro-caledonicus	0.19	53.20	40.81	0.17	4.23	0.03	0.87	0.50
P. douarrei	0.42	64.0	27.58	0.40	4.20	0.04	1.80	0.56

TABLE IV-5

Percentage of Water-Soluble nickel on dry weight basis.

	Total (ug/g)	Percentage	
		H_2O,0°C	Residue
H. kanaliense:			
Freese-dried	7300	56.20	43.80
Fresh leaf	7200	78.0	22.0
P. suteri			
Freese-dried	190	52.63	47.36

(ii) Differential contrifugation

This technique was employed in an effort to determine the subcellular distribution of nickel within the leaf tissue and to observe whether the nickel had any preferential binding sites in the cell.

(a) Method

As this was a preliminary study it was not thought necessary to experiment for optimum conditions for the separation of the various cell organelles. The gravitational forces similar to those given by Stern (1968) were used. Several washings and the use of a simple optical microscope ensured fractions were of reasonable purity.

A 1g sample of freeze-dried H. kanaliense leaf tissue (6,000 ug/g Ni) was homogenated for 60 secs in 10 sec bursts with 20 cm^3 of 0.4 M sucrose and 0.025 M phosphate buffer at pH 6.8. A Waring blender was used at top speed with short

intervals between bursts to prevent excessive temperature rises. All operations were carried out as close as possible to 0°C. The resulting pulp was filtered through a 120 micron nylon mesh and the liquid centrifuged to give the five fractions listed in Table IV-6.

A 'SORVALL' superspeed RC2-B automatic refrigerated centrifuge (rotor of radius 4.25) was used except where speeds were greater than 20,000 rpm, as was the case in separating microsomes. An ultra-centrifuge with a high speed titanium rotor was used in that case.

Fractions were taken to dryness at 80°C, ashed at 450°C to oxidize organic material and the residue taken up in 2M hydrochloric acid. Nickel was analysed by atomic absorption.

(b) Results and discussion

The average of two replicates of a representative freeze-dried leaf sample are reported in table IV-6. Precision was better than ± 20 percent. Nickel quantities are given as a percentage of total amount in tissue.

TABLE IV-6

Distribution of nickel in various differential centrifugation fractions.

Fraction	Centrifugation			Percent Ni
	g	r.p.m.	min	
Residue	-	-	-	15.50
Cell wall, debis	112	1000	5	25.96
Chloroplasts	1085	3000	10	2.12
Mitochondria	10,200	13,000	12	0.61
Microsomes	110,000	30,000	75	0.1
Supernatant	-	-	-	55.60

Over half of the nickel remained in the supernatant after high speed ultra-centrifugation. However if account was taken of the dry weight of each fraction, it was seen that nickel was evenly distributed among all the fractions. Kelly et al (1974) confirmed this with similar studies on Hybanthus species. Uniform distribution of nickel within leaf tissue of all species examined, was established. Gambi (1967) however found preferential localization of nickel in the stems of Alyssum bertolonii. The red nickel dimethyl-glyoxime complex showed a very intense colour in the sclerenchymatous areas placed between the vascular bundles.

Freeze-drying itself is a process which causes extensive rupturing of cell components, but fresh leaves were not available for study. Severne (1972) showed that results obtained from fresh leaf tissue of Hybanthus floribundus were considerably different to those obtained from freeze-dried material. He found 92 percent of the nickel remained in the supernatant. It was apparent that most of the nickel in the cell wall and debris was due to the freezing drying process. This could not be confirmed however.

Overall, however, it seems that the above results are in reasonable agreement with the solvent extraction experiments and suggests that most of the nickel in the leaf occupies a cytoplasm or vacuole location and is possibly a small polar or ionic compound. Ernst (1972) found heavy concentrations of nickel in the cell sap from Dicoma niccolipera. Cobalt and chromium were not detected in the cell sap of the leaves and lead in only small quantities. He states

that it was evident that only part of the various heavy metals was localized in the vacuole system, and other parts of the cell must also be involved in accumulation. Turner (1970) showed that a great part of the zinc and copper was deposited in the cell wall of some European heavy-metal accumulating plants.

C. INVESTIGATION OF ALCOHOL-SOLUBLE EXTRACTS

1. Introduction

One possibility that was considered during earlier work was that nickel may in fact be chelated to an amino acid. Various amino acids are well known to complex readily with the nickel (II) ion (Refer table IV-1). Van Soestbergen et al (1973) investigating ^{63}Ni complexes in rabbit serum, using chromatography on sephadex columns, demonstrated five distinct ^{63}Ni complexes in the ultrafiltrates, and although chemical identification was not achieved, they noted that one fraction resembled nickel-histidine. Peterson (1969) studied the distribution of zinc-65 in species of Agrostis and investigated an anionic complex of ^{65}Zn. It was thought that zinc was probably bound to aspartic and glutamic acid carbonyl-groups, and also to the imidazole group of histidine as shown by Broda (1965). Peterson and Butler (1971) describing a toxic seleniferous plant reported the occurrence of selenocystathionine. Other seleno-amino acids occur in other plants (Virupaksha and Shrift, 1963; Peterson and Butler, 1967).

An investigation of amino acid occurrence is also of interest from a taxonomic point of view (Peterson and Harris, 1970; Dunnill and Fowden, 1965). Characteristic and very reproducible amino acid patterns have been obtained for some

Hybanthus species and other nickel accumulators by Kelly et al (1974).

2. Sample preparation for amino acid Analysis

The three nickel accumlators, H. kanaliense, P. douarrei and P. suteri were studied and compared.

Preliminary work involved extraction of amino acids from freeze dried material with 70 percent ethanol, but a methanol-chloroform-water (MCW) solvent system proved to be more efficient (Bieleski and Turner, 1966).

One g of powdered freeze-dried leaf tissue was extracted with 10 cm^3 of MCW (12/5/3 v/v) for 10 min at 0°C. The homogenate was centrifuged, and the supernatant collected. This procedure was repeated and the MCW extracts combined. To this was added 5 cm^3 of chloroform and 5 cm^3 of water. The resulting two phase system was centrifuged and the chloroform layer discarded. The aqueous layer containing the amino acids was reduced in volume and absorbed on to a column of Zeo-Karb 225 (H^+), or Amberlite IR-120, polystyrene cation exchange resin, equilibrated with 70% ethanol. The amino acids were eluted with 30 cm^3 of 25 percent ammonia and the amino acid fractions were reduced to 1 cm^3 on a rotating evaporator under vacuum at 35°C. This process was necessary to remove pigments, sugars and other bulky neutral compounds so that samples could be concentrated sufficiently for electrophoretic examination, and so that tailing and other over-loading problems could be avoided.

3. High voltage Electrophoresis

(i) Methods

Paper electrophoresis was performed

on a high-voltage apparatus described in section II-B-4. Experiments were carried out at both pH 2.1 and pH 6.5 to obtain optimum conditions for separation of the individual amino acids. Paper chromatography in the second dimension was not deemed necessary in this investigation although for more detailed patterns, it would be required.

Concentrated extracts were applied to the paper using a glass microlitre pipette at loadings of 10 to 50 ul per 2 cm^3 streak. The paper used was Whatman No. 1, 46 x 57 cm, chromatographic paper. The origin was placed 20 cm from the bottom of the sheet for runs at pH 6.5. Buffer was spread evenly over the paper, so that the solvent met at the origin, and blotted dry. Electrophoresis was carried out for 40 min at a voltage of 3 kV. The current varied depending on the width of paper used. Marker solutions containing a number of standard amino acids were run concurrently with all samples. Papers were dried overnight.

The run at pH 6.5 separated out the acidic and basic amino acids which migrated towards the positive and negative electrodes respectively. The neutrals moved slightly towards the negative electrode. The development of marker solutions showed the extent of this shift so that they could be cut out, sewn on to a fresh piece of paper and run at pH 2.1. These amino acids ran towards the negative electrode to varying extents.

Amino acid spots were developed using ninhydrin (0.2% in acetone) with 1% collidine. The specific colour reactions of this developer facilitated identification. In some cases papers were developed with 1% ninhydrin

cadmium nitrate in acetone (17/3 v/v). This reagent was more sensitive but lacked the colour specificity of the ninhydrin-collidine.

The identity of the amino acids was established by running standards and comparing them in the electrophoretograms, and also by specific colour reactions.

Nickel was located using a 0.2 percent α-furil dioxime spray which is specific for nickel and has a very high sensitivity. Dimethylglyoxime is also suitable but has a lower sensitivity; approximately five times lower than the α-furil-dioxime (Sandell, 1959).

(ii) Results

Figs IV-2 and IV-3 show the amino acid distributions for Pimelea suteri, Psychotria douarrei and Homalium kanaliense. The movement of nickel is also shown in fig IV-2 at pH 2.1 the nickel complexes seem to have been cleaved, as all nickel migrated the same distance as a nickel aqueous complex. These movements were off the map shown in fig IV-3 in the direction of the negative electrode.

The amino acids in these two figures were identified as : (1) ethanolanime, (2) unknown, (3) α-amino-butyric acid, (4) lysine and/or Arginine (5) histidine (6) various neutral amino acids (see fig IV-3), (7) glutamic acid, (8) aspartic acid, (9) unknown which migrates the same as histidine at pH 6.5, and could possibly be a peptide, (10) glycine, (11) alanine, (12) valine and isoleucine (serine also possibly present), (13) threonine, (14) proline, (15) methionine, (16) phenylalanine, (17) Hydroxyproline and (18) cysteine.

Aspartic and glutamic acid were the dominant acidic acids and were easily recognised. Nickel was not associated with either of these. Note that the basic and acidic amino acids at pH 6.5 were present in all cases and differences were only seen in the "neutral" amino acids which migrate as positively charged species at pH 2.1. Alanine was present in large quantities in all three species but H. kanaliense was lacking in hydroxyproline, and proline. Both were found in substantial quantities in P. douarrei and P. suteri although these plants were characteristically different in nature.

No significant quantities of nickel were observed to be associated with any of the amino acids although the formation constant for a nickel histidine complex is quite high (log K, K_2 = 15.9). It must be pointed out that the extracts used here were alcoholic and quantities of nickel extracted were only moderate. Behaviour however was the same as for the water-soluble extracts in which much larger quantities of nickel occurred. Amino acid composition of the three species could not be related to nickel concentrations although total nickel differed greatly among the three (P. suteri, 200 ug/g; H. kanaliense 7,000 ug/g, and P. douarrei, 25,000 ug/g of dry tissue).

Other amino acids may have been present, but if this were so they could not be observed and they must have been present in fairly low quantities or were differentially adsorbed during the extraction processes. For example, arginine, because of its strong basic tendencies may be differentially adsorbed to leaf tissue (Bieleski and Turner, 1966).

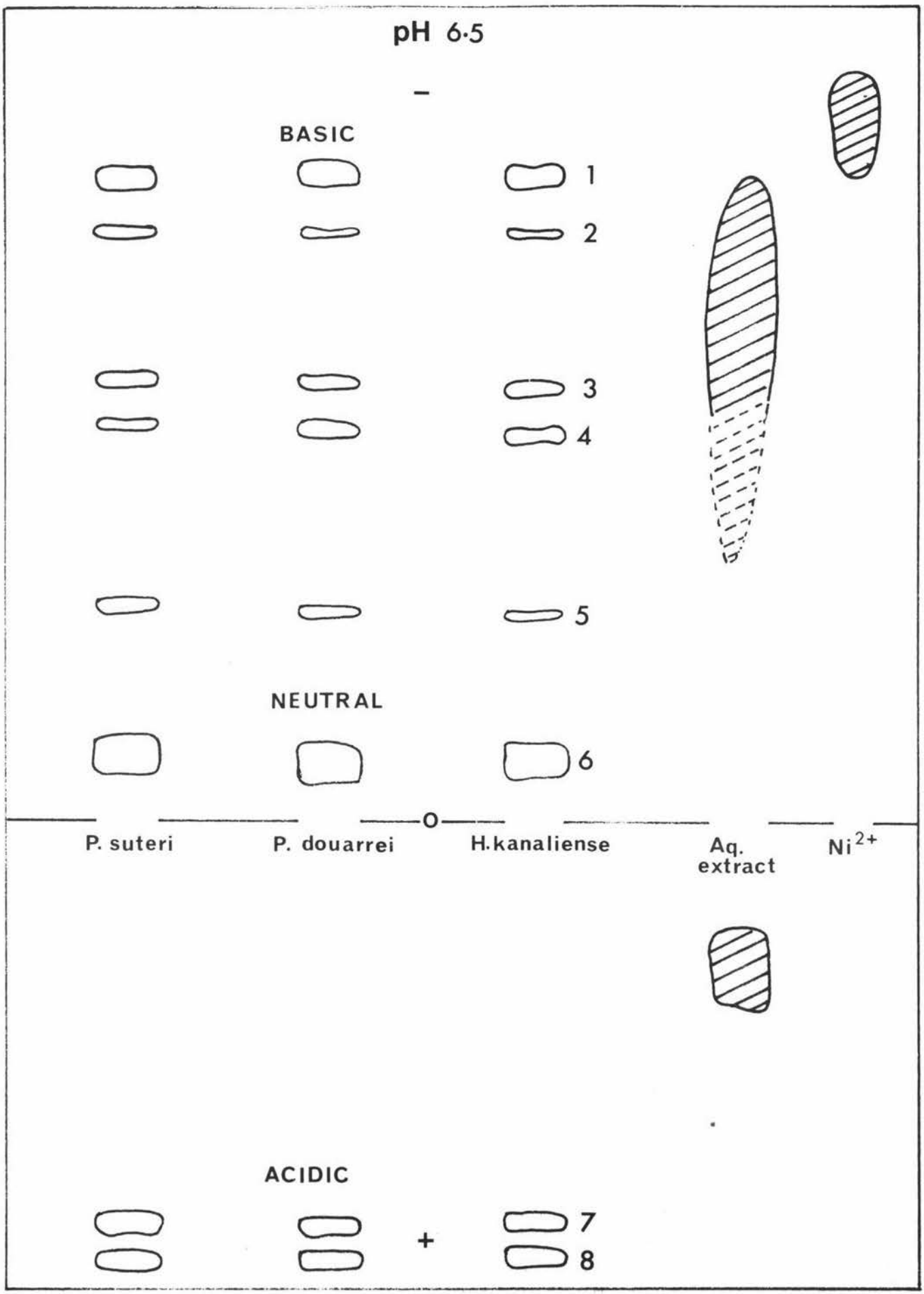


FIG.IV-2 Diagram of an electrophoretogram showing nickel and amino acid distributions at pH 6.5

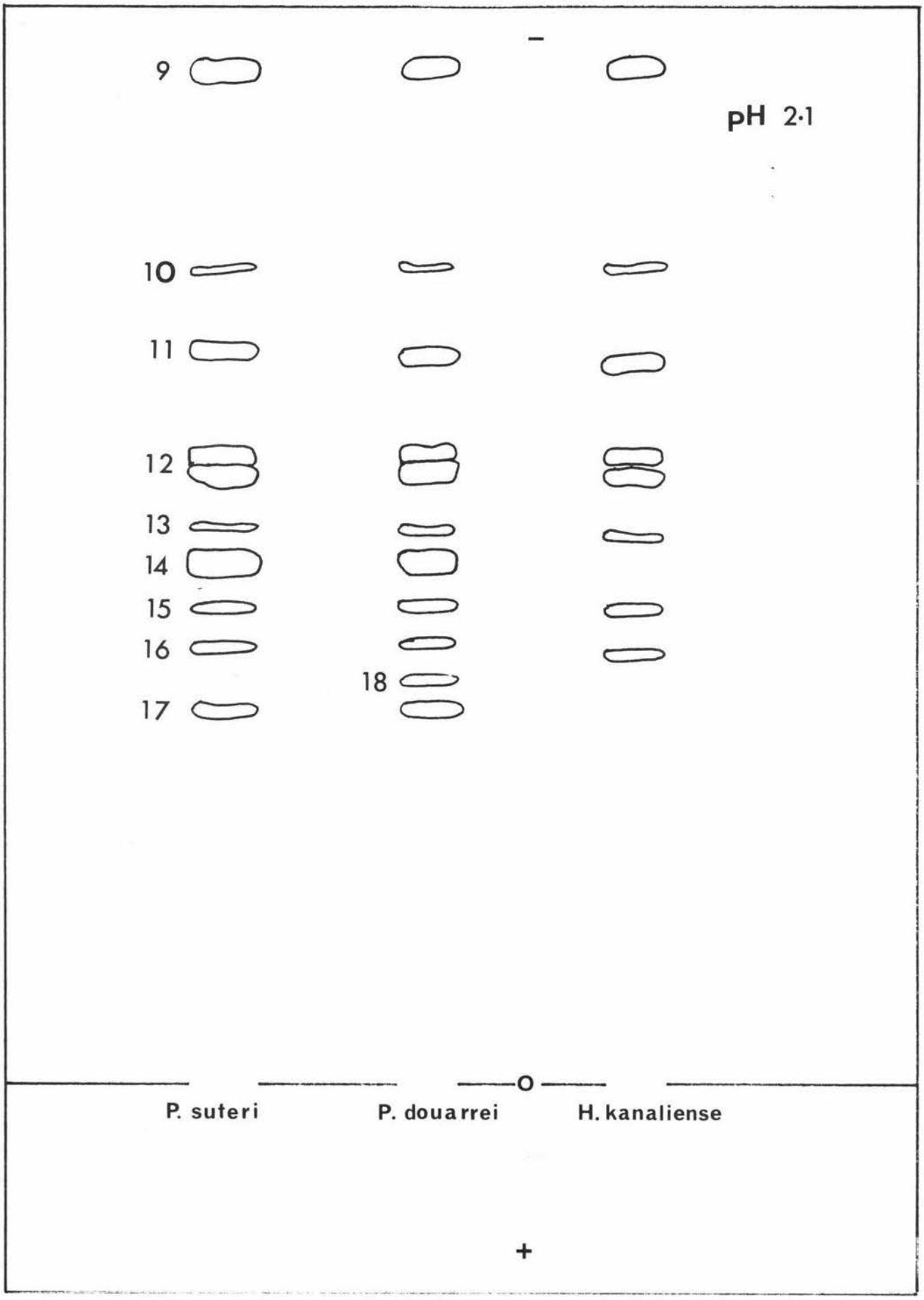


FIG. IV -3 Diagram of an electrophoretogram showing distribution of nuetral amino acids at pH2.1

D. INVESTIGATION OF THE WATER-SOLUBLE EXTRACT

For preliminary work 1 g samples of H. kanaliense were taken and extracted with cold distilled water as previously described. Chloroform was used to extract pigments and lipids from the aqueous phase. The nickel-containing solution was reduced to a small volume on a rotary evaporator at 35°C and filtered. This procedure resulted in a 1 cm^3 solution containing approximately 4,000 ug of nickel. For preparative work, larger quantities of freeze dried material were taken or several extracts were combined.

This water soluble extract was used for all further work.

1. Electrophoresis

As electrophoresis sorts material according to charge and to a lesser extent to molecular size, this technique can give some information about the possible nickel complex(es). High-voltage paper electrophoresis has been used by many workers in the study of metal mobilities and in the separation of biological materials (Bremner and Knight, 1970; Peterson 1969; Timperley, 1971; Tiffin 1967, 1966, 1971; and Effron, 1960).

(i) Method

The electrophoretic conditions used are described elsewhere. A 10 ul sample of the crude nickel-containing water extract was applied to Whatman No. 1 paper as a 1 cm streak. A standard hexa-aquo nickel complex was also applied on the same paper. Identical papers were run at pH 2.1 and pH 6.5 for 40 min at a voltage of 3 kV. Papers were air dried and the nickel

located using -furil dioxime. Because of the possibility that some nickel may not have shown up by this method, the papers were cut into 2 cm strips which were ashed and the nickel content analysed by atomic absorption.

(ii) Results

Fig IV-4 shows the analyses of the electrophoretograms at the two different pH values. (A, pH 6.4 and B, pH 2.1).

The results indicated a different behaviour of the nickel compound in the extract at different values of pH. At pH 2.1, the nickel complex was cleaved as its mobility was the same as that of the nickel (II) standard. The run at pH 6.5 however showed more complex behaviour. The scan indicated the presence of both negative and positive complexes. The amount going towards the positive electrode moved fairly compactly whilst the positively-charged species exhibited trailing effects.

The atomic absorption results confirmed the pattern seen by development with ∝-furil dioxime. Tiffin (1971) found a similar pattern in carrot exudates.

The movement of nickel was seen to be the same as that from an alcoholic extract studied previously. It must be pointed out also that the behaviour of nickel varied with concentrations although varied concentrations did not effect the movement of the divalent nickel aqueous standard. The results indicated that larger groups on the nickel from the plant extract may have retarded its movement compared with the standard nickel. It may have been interesting to observe the behaviour at a more alkaline pH. It would not be surprising to see larger quantities more

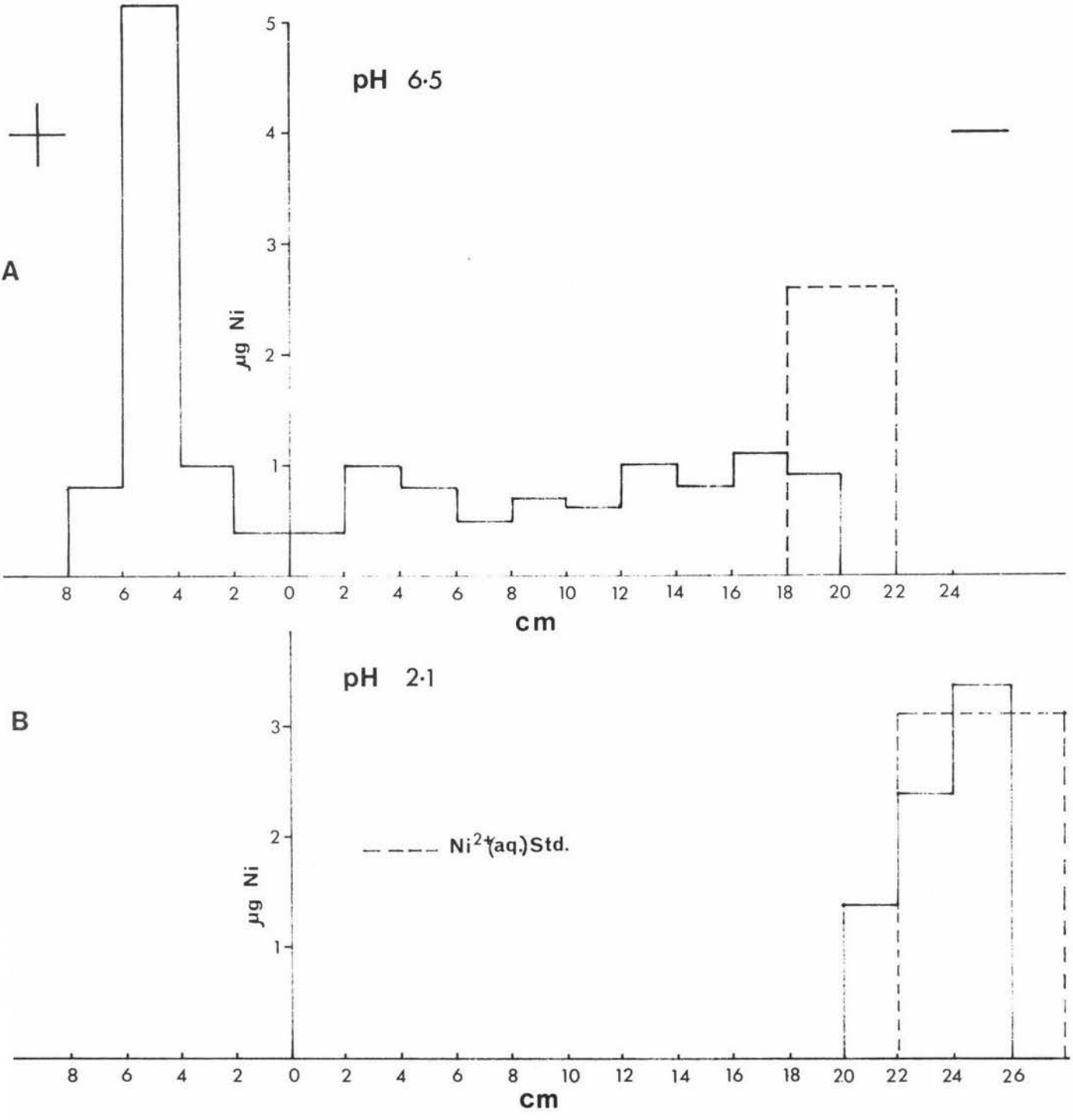


FIG.IV-4 Electrophoretic distribution of nickel from leaf extract of H. kanaliense.

towards the positive electrode. This was not done because of the impracticality of switching large amounts of buffer from the electrophoresis tanks.

Electrophoresis was also attempted on a preparative scale but large enough quantities of nickel complex could not be efficiently isolated from crude preparations. Gel filtration was therefore used as discussed below.

2. Column Chromatography

Gel filtration on sephadex columns appeared to be the best method for the isolation and purification of the nickel compound(s). The technique is technically simple to perform and is very inert to composition of the eluant and temperature. Very labile compounds may be treated with little risk of decomposition. The procedure may be used under very mild conditions. Sephadex gels are available with varying gel matrixes so that the fractionation range may be greatly varied. Gels are very stable chemically, with a low content of ionic groups. It is impossible to avoid charged groups completely. The commercial gels used in this work are manufactured under the trade name Sephadex and are dextrans.

The Sephadex powder obtained, was swollen in distilled water overnight before any column packing. Once columns are packed they may be used many times due to the extreme stability of the gel and contaminants may be easily removed with strong alkali.

Sephadex G-10 with a working range from 100 to 700 molecular weight was used as the nickel complex was suspected of being of low molecular

weight; probably between 200 and 400. The nickel (II) aquo complex has a molecular weight of 167.

(i) Method

A column (60 cm x 2.5 cm) was packed with water-swollen G-10, washed with 0.1M hydrochloric acid and equilibrated with water. Both H_2O and 0.05 ammonium acetate were used as eluates with slightly different effects. A detran-blue dye of molecular weight 2,000 was used to check the functioning of the column and establish the void volume (65 cm^3). Compounds with molecular weight greater than 700 would be eluted at this volume, whilst others would be successively retarded in the column the lower the molecular weight became.

A small quantity (1 cm^3) of concentrated aqueous extract, containing up to 10 mg nickel (perhaps 50 mg of complex) was applied evenly to the column head and fractions collected with an automatic fraction collector. 4 or 6 cm^3 fractions were obtained at a flow rate of about 0.3 cm^3/min.

Every second fraction was analysed for nickel by atomic absorption. Better separation was achieved by recycling the nickel-containing fractions. This effectively increased the column length.

(ii) Results

The elution patterns for the nickel-containing fractions are shown in fig IV-5. Nickel fractions come very close to the solvent front marked by the elution of high molecular weight brown coloured fractions. Retardation of the early fractions was not very great but Spitzey et al (1961) have reported that sephadex

gel repels anions which would therefore be carried with the solvent front. Electrophoresis has already indicated that a proportion of the total nickel is in the form of a negative species.

The use of 0.05 ammonium acetate as the eluant shows a fractionation of two distinct species. The latter fractions may contain the aquo complex of nickel as these coincide with the elution of a nickel (II) aqueous standard. A higher ionic strength would probably have given better resolution. The effects of a higher ionic strength on the nickel complex, were unknown however. Distilled water was finally chosen, although tailing is more pronounced than at a higher ionic strength. Dilution from tailing was overcome to a certain extent by successive recycling of the nickel fractions. The use of distilled water also made identification experiments easier. Volatile electrolytes are difficult to remove completely and may interfere in subsequent work.

Fig IV-5A shows the elution of the intense green band of nickel compound(s) after three successive trips through the column. This clear green solution was eluted between the brown coloured matter at the solvent front and a light yellow band of smaller molecular weight material containing amino acids, organic acids etc.

The partially-purified nickel fractions were reduced in volumn on a rotatory evaporator at 30°C. Ultraviolet, visible and infra-red spectrophotometry were conducted on this solution before reducing to dryness and washing with ethanol.

The molecular weight of the complex(s) estimated from the relationship between elution

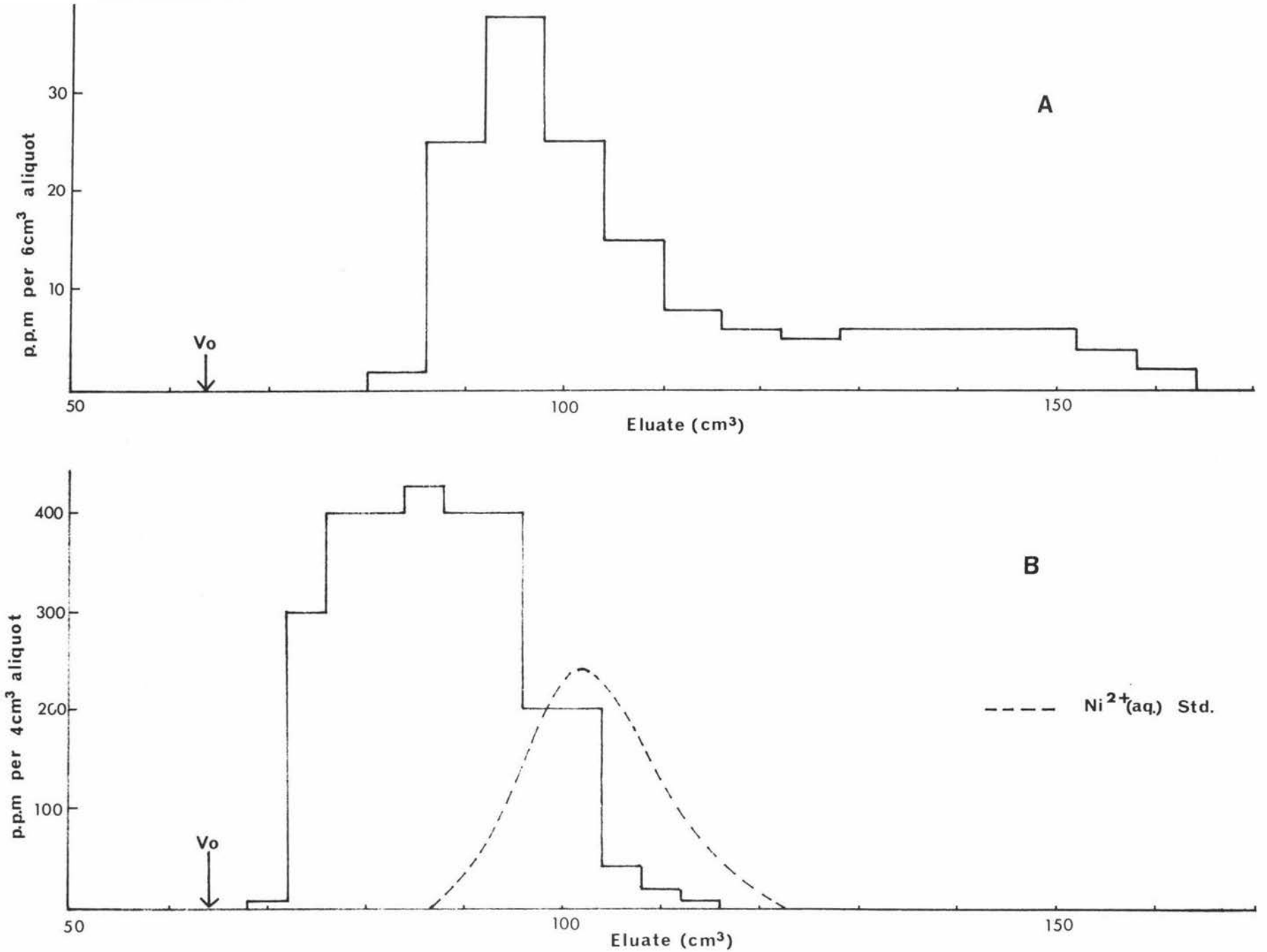


Fig. IV-5 Elution pattern from a Sephadex G-10 column showing distribution of nickel.

behaviour and molecular properties was in the range 250 to 450. This estimation was based on data from "Sephadex G-10 and G-15" by Pharmacia Fine Chemicals. Because of the large spread of the nickel fractions the range could not be narrowed down further than this.

3. Characterization of nickel compound

(i) Spectrophotometry

U.V. spectrophotometry was carried out on a purified aqueous extract obtained from the G-10 column. A Shimadzu MPS-5000 spectrophotometer with a scan range of wavelengths from the ultra-violet into the infra-red part of the spectrum (180 nm - 2,500 nm) and a setting of 0-1 absorbance was used. The spectrum obtained is shown in fig IV-6 along with that of the nickel (II) aquo species. Octahedral nickel complexes are well known for their very low molar absorbances. Bands at 500-800 nm have molar absorbances at peaks of only 1-10 (Cotton and Wilkinson, 1966). The fact that a good response was obtained, points to the reasonably-high concentrations of complex present. The peaks are not shifted appreciably from those of the aquo complex and this shows that a considerable number of oxygens must be co-ordinated around the nickel. If nitrogen were co-ordinated, the peaks would have shown much greater shifts to lower wavelengths. Amine complexes e.g. $(Ni\ (en)_3)^{2+}$ characteristically have blue or purple colours due to the shift in the absorption bands towards the red (Cotton and Wilkinson, 1966). The biggest difference between the two spectra shown in fig IV-6 is the change in peak shape for the absorption band centred at 700 nm and the difference in the absorbance.

Both solutions contained similar quantities of nickel. Pestemer et al (1949) observed that in the absorption spectra of the monobasic carboxylic acid salts of nickel (AcOH, $EtCO_2H$, $PrCO_2H$), the bands of the nickel (II) ions and the anions persist throughout but for the salts of the polybasic acids (oxalic, tartaric and citric), stronger shifts due to complex formations appear in the first and third absorption bands. Bobtelshy and Heitner (1951) showed that the effect of the citrate is to increase the absorption of the cation.

Infra-red spectrophotometry was also undertaken with a small amount of the crystalline complex ground with Nujol. The resulting spectrum, a scan from 4,000 cm^{-1} to 300 cm^{-1}, showed the domination of the two water stretching bands at 3,400 cm^{-1} and 1,600 cm^{-1}. It is quite likely that both co-ordinated water and water of crystallization may be involved. Other prominent peaks were at 1,540 cm^{-1}, 1,075 cm^{-1}, 900 cm^{-1}, 845 cm^{-1} and 722 cm^{-1}. The large absorption of the water at 1,600 cm^{-1} obscured possible carbonyl stretching frequencies in this region. Nakamoto (1970) gives band assignments for the complex. $K_2(Cu(OX)_2)\ 2H_2O$ as (C=O) at 1720 cm^{-1}, 1,672 cm^{-1}, and 1645 cm^{-1}; (C-O) at 1,394 cm^{-1}, 1,245 cm^{-1} and 893 cm^{-1}, and (M-O) at 818 cm^{-1}, 556 cm^{-1}, and 417 cm^{-1}. Little could be concluded from the spectra obtained except that they showed the presence of (C-O) and (C=O) stretching. Small peaks at 845 cm^{-1}, 722 cm^{-1} and 600 cm^{-1} may be attributed to (Mi-O) stretching, although this is speculation.

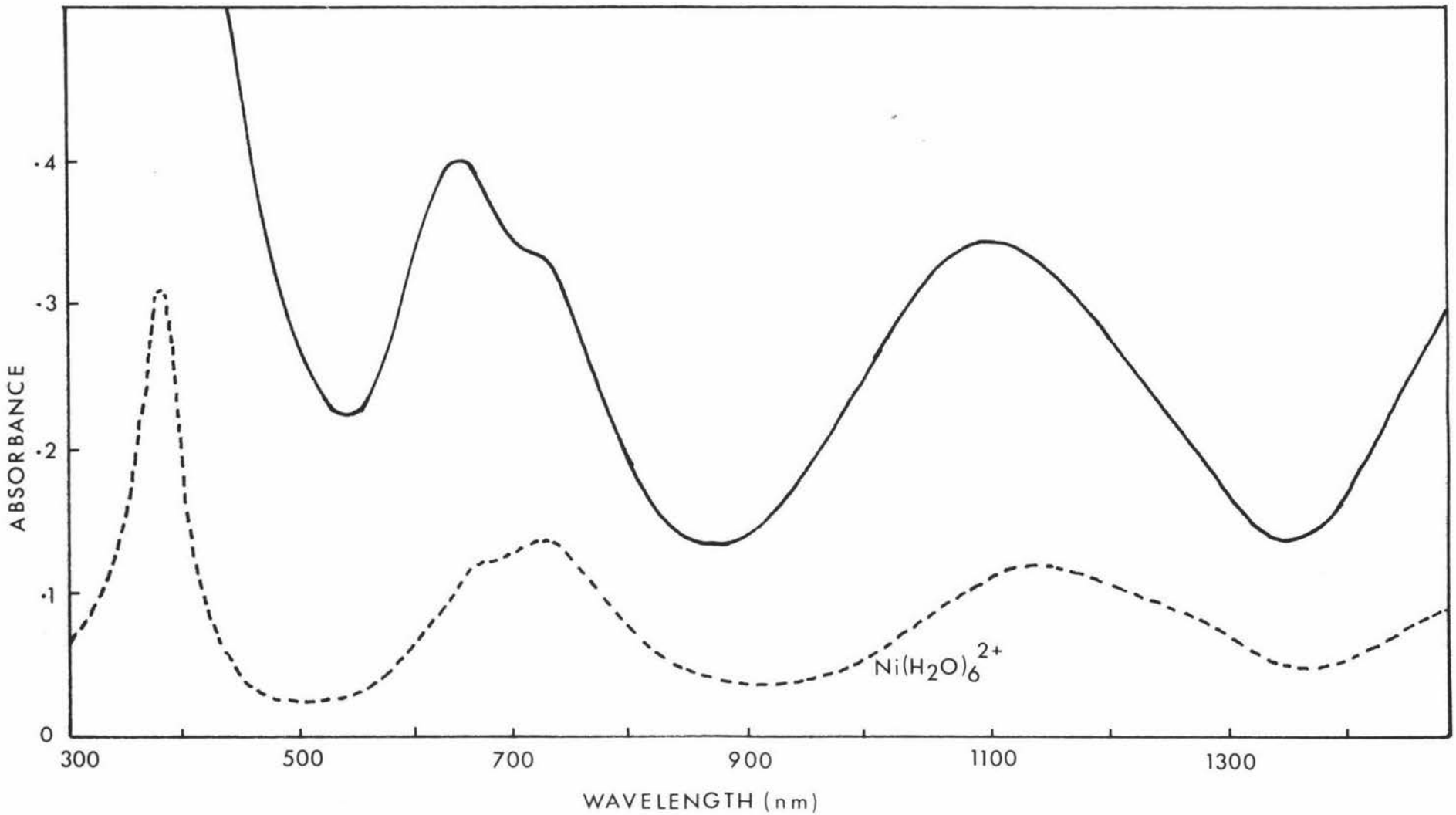


Fig. IV-6 Absorption spectra of nickel complex (——) and nickel hexaquo ion (----).

(ii) Analysis for carbon, hydrogen, nitrogen and oxygen.

A small quantity (25 mg) of crystalline complex was analysed for carbon, hydrogen and nitrogen. Unfortunately oxygen could not be determined in the presence of nickel. Nickel was analysed by atomic absorption spectroscopy and sodium and potassium by flame photometry. Results obtained were as follows : Carbon, 28.09 percent; hydrogen, 4.73 percent; nitrogen, 0.73 percent; nickel, 8.31 percent; potassium, 3.0 percent and sodium, 4.0 percent. The small quantity of nitrogen present is from impurities. The amount of oxygen is probably close to 50 percent.

(iii) Mass spectrometry

Nickel was released from its ligands by bubbling hydrogen sulphide through a aqueous solution of the complex. Nickel sulphide was precipitated in the presence of ammonia fumes. The black precipitate was filtered off. The clear solution was then taken to dryness on a rotary evaporator. Microgram samples of the resulting glass-like substance were analysed using a A.E.T. M.S. 902 mass spectrometer.

(iv) Spot tests for specific groups

Various spot tests (Feigl, 1966) were carried out to determine the presence of certain functional groups. Tests were carried out for dibasic and polybasic acids, hydroxy acids and simple reducing carbohydrates. To detect this last class, Tollens reagent was used. This involves the reaction of $(Ag(NH_3)_2)^+$ and OH^- ions with the reducing compound to produce silver as a black precipitate. A very intense response was obtained.

Slightly positive tests were obtained for citric and malic acids. This involved the observation of fluorescence after oxidation. However the fluorescence was not comparable with the intensity obtained from standards. Due to a certain degree of non-specificity, caution must be taken, when interpreting the results. Mass spectrometry showed that citric acid was unlikely to be present in the sample.

4. Results and Discussion

Spectrophotometric work has indicated the dominance of oxygen co-ordinating ligands on the nickel. The absence of any significant amounts of nitrogen confirms this. Carbon and oxygen are also known to be present in large amounts. As the nickel complexes are retained on a sephadex G-10 column the molecular weights are below the exclusion limit of 700. Retardation on the column indicated a weight somewhat below this value. Movement on chromatograms and electrophoretograms showed the nickel to be associated with various unknown organic acids.

The importance of organic acids in plant metabolism is well established (Thimann and Bonner, 1950). The presence of carbonylic acids such as analic acid and the hydroxy acids, malic, citric and tartaric, have been investigated in various plants. (Rasmussen and Smith, 1961; Hulme and Richardson, 195 , and Palmer, 1955). Cooil (1948) found that the higher total acids associated with high potassium in expanding guayule leaves was largely accounted for by high citric acid levels whereas in nature leaves high malic acid levels were associated with high calcium levels. Ritter-Studnickce (1972) compared

a number of plant species which occur on serpentine and calcium-rich soils and found that the amount of total acids was higher in serpentine plants and production of organic acids appeared to be stimulated by magnesium accumulation. Percentage potassium in dry leaves of *H. kanaliense* was 2.40 whilst levels of calcium and magnesium were found to be 0.93 and 0.24 percent respectively.

It is known that oxalate, citrate and other salts of polybasic acids are capable of complexing with nickel. Stability constants have been assessed by various workers (Patnaik and Pari, 1957; Li *et al*, 1959). Migal *et al* (1958) have shown the order of stability of various citrate complexes as Cu > Ni > Co > Zn > cd. In a spectrophotometric study of complexes composed of citrates or tartrates of nickel, cobalt, copper or chromium, Bobtelsky and Heitner (1951) showed that the effect of citrate was to increase the absorption of the cation. Peak positions were not greatly altered.

Electrophoresis of the nickel extract from *H. kanaliense* showed that at least three complexes existed, one of which is probably the nickel aquo complex which with the lowest molecular weight migrates the furthest. Further separation of these complexes could not be achieved on a sephadex column. This pointed to the likelihood of some exchange process occurring. Tiffin (1971) found at least two forms of nickel in tomato exudate, the relative quantities of each depending on total nickel translocated. At physiological levels of nickel in the xylem, he found it to be translocated as a negatively charged species.

Tiffin (1967) has investigated the role of citrate as a carrier of iron and possibly of other cations. He did not, however, identify the nickel carrier. Kovorin (1952) showed from experimental data that nickel citrate is more stable than iron citrate.

The movement of the anodic nickel component was quite compact indicating its stability compared to the extensive trailing of that moving cathodically. The strongly-chelated aquo complex moved as a compact spot towards the cathode. Tiffin (1971) observed similar behaviour of nickel in tomato exudates.

Mass spectrometry however did not confirm the presence of citrate. The spectrum obtained did not compare with that of citric acid. Also, owing to the insolubility of the complex in alcohols, hydroxy-acids could be fairly safely ruled out as the chelating agent. Citric and other similar acids are present in many plants but because of the unique and enormous accumulation of nickel by some of the plants mentioned in this thesis, it seemed likely that a more specific and perhaps unusual chelating group was involved. Tests and behaviour so far seem to indicate the acid of a simple sugar. Some of these compounds such as D-gluconic acid, are insoluble in alcohol, benzene and ether as was observed with the nickel complex. The molecular weight of gluconic acid is 196. If two of these were complexed to the nickel through the oxygens on the carboxylic group, a complex of approximately the correct molecular weight and composition is obtained. Some water, as shown by infra-red spectra, is also associated with the nickel.

There is some evidence that the chelated ligand is a simple sugar-acid but conclusive proof will require further work which is out of the scope of this study.

GENERAL

CONCLUSION

In view of the specialized nature of the sections in this thesis, no attempt will be made to discuss further the results obtained: rather some of the findings will be summarised.

This thesis had two objectives. The first one was aimed at elucidating some of the soil factors that influence the distribution of serpentine flora. A statistical approach using multivariate methods was used in distinguishing between soil sites supporting endemic species (Pimelea suteri and Myosotis monroi) and those supporting non-endemics (Cassinia vauvilliersii, Hebe odora and Leptospermum scoparium).

The second objective was an investigation into the chemistry of nickel in the New Caledonian nickel accumulator, Homalium kanaliense. Although the form of nickel in the leaves of this plant was not positively elucidated it is hoped that some useful ground work has been covered. Some idea of its nature in the plant has been obtained, and some possible chelates can be ruled out.

In conclusion, the specific findings and accomplishments of this project were :

(i) The characterisation of five soil groups on the basis of chemical composition using discriminant analysis, and from this the tendancy of the two endemic plants to occupy localities of highest magnesium concentration was shown.

(ii) Magnesium is by far the best discriminator, with nickel the second best. Other elements provide little more discrimination than would be expected from a completely random plant distribution.

(iii) The endemic plants may be characterised by an ability to survive in areas where the availability of the already low calcium level is decreased by the presence of large concentrations of readily-extractable magnesium.

(iv) There is little evidence that either the New Zealand or New Caledonian plants restrict uptake of nickel.

(v) The New Caledonian species contained higher-than-average contents of the nutrients, calcium, phosphorous and potassium in spite of the particularly low concentrations of these elements in New Caledonian serpentine soils. The very high uptake of nickel may be linked to the concomitant accumulation of nutrients.

(vi) The observations of the stimulating effect of nickel on uptake of calcium, phosphorous and potassium by the New Zealand plant Pimelea suteri.

(vii) The possible metabolic role of nickel is strengthened by the observation that this element is not excluded from the reproductive and highly metabolising organs of some of the plants.

(viii) The demonstration that nickel in Honalium kanaliense leaves is chelated to a highly water soluble, (insoluble in organic solvents), organic compound. Limited evidence points to a simple sugar-acid.

(ix) Nickel toxicity is alleviated by chelation.

The author maintains that the objectives of this study were, on the whole, fulfilled, when the limited scope of the work is considered.

Some additional knowledge has been put forward as regards the somewhat enigmatical problems of serpentine flora. It is hoped that near future work will positively identify the chelating species of the nickel in some of these plants.

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