TRACE ELEMENTS

IN

NEW ZEALAND PLANTS

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ABSTRACT

In biogeochemical studies initiated for the first time in New Zealand, some trace elements in indigenous plants and in the soils supporting these plants, were determined by emission spectrography and atomic absorption spectrophotometry.

A test of the biogeochemical method of prospecting was made by studying elemental concentrations in leaves of three tree species and in the corresponding soils from mineralised and non-mineralised ground at Copper-stain Creek, North-West Nelson. All samples were analysed for copper and molybdenum. Olearia rani was also analysed for zinc.

Elemental contents in plants and soils were compared by correlation calculations on a computer. The molybdenum content of the ash of O. rani leaves showed a highly-significant correlation with the content of the same element in corresponding soil samples. This indicated that O. rani could be used as a biogeochemical indicator for molybdenum mineralisation. No other significant plant-soil correlations were found for molybdenum, copper or zinc.

For a further set of O. rani samples, leaves, twigs, wood and flowers were all analysed for zinc, copper and molybdenum. This data showed that the leaves of O. rani were better than the other parts of the plant, and that analyses based on ash weight were better than dry weight values for indicating molybdenum mineralisation in the soil.

Cumulative frequency diagrams gave values for threshhold concentrations used to delineate the anomalous areas at Copperstain Creek.

Molybdenum contents in the ash of plants showed wide variations, ranging from one to 1600 parts per million. Copper and zinc contents showed less variation but there were significant differences in the mean values for each species.

A New Zealand serpentine flora and the associated soils from near Dun Mountain on the Nelson Mineral Belt was studied. In an initial orientation survey, it was found that seventy-one samples of twenty-six species showed that wide variations existed in the concentrations of the elements chromium, nickel, cobalt and copper. Six of these species were sampled further and analysed for chromium, nickel, copper, cobalt, calcium and magnesium.

The species Cassinia vauvilliersii, Hebe odora and Leptospermum scoparium were sampled both randomly and from a localised area of serpentine where soils were more uniform. Plant elemental contents were found to vary up to several orders of magnitude in both sets of samples. Specimens of the same species sampled from near the boundary of serpentine with sedimentary rocks and from an andesitic area at Mt. Egmont, contained much lower amounts of chromium, nickel, cobalt and magnesium and higher amounts of calcium than samples from serpentine.

Correlation coefficients were calculated for the relationships between pairs of elements and showed that for C. vauvilliersii, there were highly-significant correlations between plant ash and soil contents for chromium, nickel and cobalt. H. odora and L. scoparium showed similar but less pronounced correlations for the same three elements. It was concluded that these species, especially C. vauvilliersii would be useful for biogeochemical prospecting.

Twenty samples of each of the serpentine-endemic species, Myosotis monroi, Notothlaspi australe and Pimelea suteri were also

collected for comparison with the other, more common species. P. suteri, in particular, is a strong accumulator of chromium, nickel and cobalt although L. scoparium also accumulates chromium to a greater degree than the other species.

The highest concentrations found included 2.6% chromium in the ash of a P. suteri and 9% chromium in a soil in which L. scoparium grew. These values are higher than any previously observed in other parts of the world. Good correlations were found for some pairs of elements showing that chromium, nickel and cobalt are strongly related in soils and in most plant species.

The range of calcium and magnesium contents in plant ash was from one to 30%. Although the exchangeable-calcium content of soils was about one twentieth of that for magnesium, the Ca/Mg ratio in plants ranged from 0.1 to 5.0.

In view of the unusual concentrations of chromium in serpentine plants, the metabolism and uptake of the radioisotope chromium-51 was studied in selected species. Translocation of ^{51}Cr when applied as chromate to cuts in the stem or the tips of branches of seedlings was observed to be greater in P. suteri and L. scoparium than in C. vauvilliersii or H. odora, as indicated by radioautography. In young L. scoparium (manuka) plants, ^{51}Cr supplied as chromate was translocated as chromate in the xylem sap as shown by high-voltage electrophoresis.

Trifolium pratense seedlings (red clover) which were more readily available and grown in nutrient solution for ease of manipulation, were able to absorb and translocate ^{51}Cr to the leaves when the ^{51}Cr was supplied as either sodium chromate or chromic chloride.

In both species (T. pratense and L. scoparium), generally much less than 5% of the ^{51}Cr appeared to be bound to protein or nucleic acids. In the roots of L. scoparium seedlings, 32% of the radioactivity was soluble in boiling 80% ethanol and a further portion was soluble in boiling water. These fractions were examined by high-voltage electrophoresis and a total of 18% of the ^{51}Cr in roots of L. scoparium cultured in nutrient solution existed as the trioxalatochromate(III) ion with lesser amounts as two other soluble anionic compounds. T. pratense did not show the presence of the trioxalatochromate(III) ion.

It was concluded that the feasibility of carrying out biogeochemical prospecting in New Zealand has been demonstrated for the first time. Further, the plant chemistry studies have contributed not only to general plant nutrition research, but also have provided basic information for the understanding of the trace element metabolism involved in biogeochemical prospecting.

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PART I

GENERAL INTRODUCTION

"In mountains in which ores or other minerals are present, growing trees usually are not healthy, that is, their leaves are pale and the trees themselves are low, bent, distorted, gnarled and rotten before reaching old age." These words of M.V. Lomonosov in 1763 probably represent the earliest written record of the effect of mineralisation on plants (Malyuga, 1964, p. 6).

Plants may be used as indicators of mineralisation in either of two ways. Biogeochemical prospecting relies on the chemical analysis of plants, while geobotanical prospecting uses the visual examination of plant species and plant morphology to indicate mineralisation.

In the last twenty years, trace element biogeochemical prospecting has been tested and found useful in British Columbia (Warren, 1962; Warren and Delavault, 1965), the United States (Cannon, 1960, 1963; Carlisle and Cleveland, 1958) and other parts of the world (e.g. Nicolls et al, 1964-5; Duvignead, 1958). However, its greatest popularity appears to have been reached in the Soviet Union where it has been used for the past thirty years. Biogeochemists are included in most mineral exploration teams in that country (Malyuga, 1964).

In New Zealand, few indigenous plants have been analysed for their trace element contents, although beech trees from the Hutt Valley (Miller, 1963a, 1963b) and exotic pine trees from Central North Island (Orman and Will, 1960) have been studied for their major element composition. Recently Wells and Whitton (1966) looked at the elemental composition of manuka from hydrothermal areas, as well as the compositions of white clover and sweet vernal, and these were compared with other samples from the Taupo area. The only previous biogeochemical study was that of Williams (1964) who reported high concentrations (600-3000 ppm) of nickel in the ash of the tree

Weinmannia racemosa over a pyrrhotite-pentlandite vein in the Takaka Valley. However, her samples from similar outcrops nearby failed to show any indication of mineralisation and this method of prospecting was therefore discontinued.

Considerable areas of land in New Zealand are at present being prospected for minerals and most of these are covered with dense indigenous forest, often with deep weathering and few rock outcrops. Biogeochemical prospecting could therefore be very useful. However, mining companies appear reluctant to use this method, as it initially requires a comprehensive orientation survey and demands more skill in collecting specimens than the routine sampling of soils and stream sediments. Another factor which has worked against the use of biogeochemical prospecting in New Zealand is the fact that the indigenous flora is unique and little use can be made of the large pool of knowledge gained in other countries for plant species of widespread distribution.

In the belief that there was clearly a place for biogeochemical studies in New Zealand, a survey of this nature was initially carried out at Copperstain Creek, North-West Nelson (Fig. I-1), where geologists and geochemists were investigating the economic potential of a sulphide deposit.

For a biogeochemical orientation survey, both plants and soils should be analysed so that a statistical comparison can be made. Williams (1964, 1967) has applied statistics to geochemical prospecting but not to biogeochemical data. Her method was an extension of that of Tennant and White (1959) who studied the distribution of biogeochemical data in addition to studies on geochemical information. However, no attempts appear to have been made to correlate soil and plant ash data. Usually plant ash

concentrations have been compared graphically with soil content (Nicolls et al, 1964-5; Cannon, 1963) or diagramatically with geological structure (Warren and Delavault, 1965) and soil content (Shacklette, 1962).

Thus much of the earlier biogeochemical prospecting work has suffered from the lack of a rigorous statistical basis for the interpretation of data, and it was hoped that such a basis could be established in the New Zealand investigations.

Ore-indicating vegetation assemblages have been known for many years. Both the calamine vegetation of zinc deposits in Europe, and the flora of seleniferous areas in North America contain suitable geobotanical indicators (Hawkes and Webb, 1960, p. 312). In some cases, the selenium-accumulating plants of Astragalus species also indicate the associated uranium and vanadium (Cannon, 1960, 1964). Many other soil types also have characteristic vegetation. Some examples are the halophytes of salt marshes, calcifuges of acid soils, and serpentine flora.

Areas underlain by serpentine or ultramafic rocks always have characteristic vegetation. The plants are widely scattered and often there are species endemic to these soils. The reason for a characteristic flora has been considered to be the elemental content of the soil (Robinson et al, 1935; Rune, 1953).

In New Zealand, there are two large areas of ultramafic rock, one in the mountains of South Westland, and the other a north-east, south-west trending belt to the east of Nelson City. Near the centre of the latter area is Dun Mountain (Fig. I-1) which is more readily accessible than most other areas. Near Dun Mountain also, are a number of disused copper and chromium mines in serpentine rock.

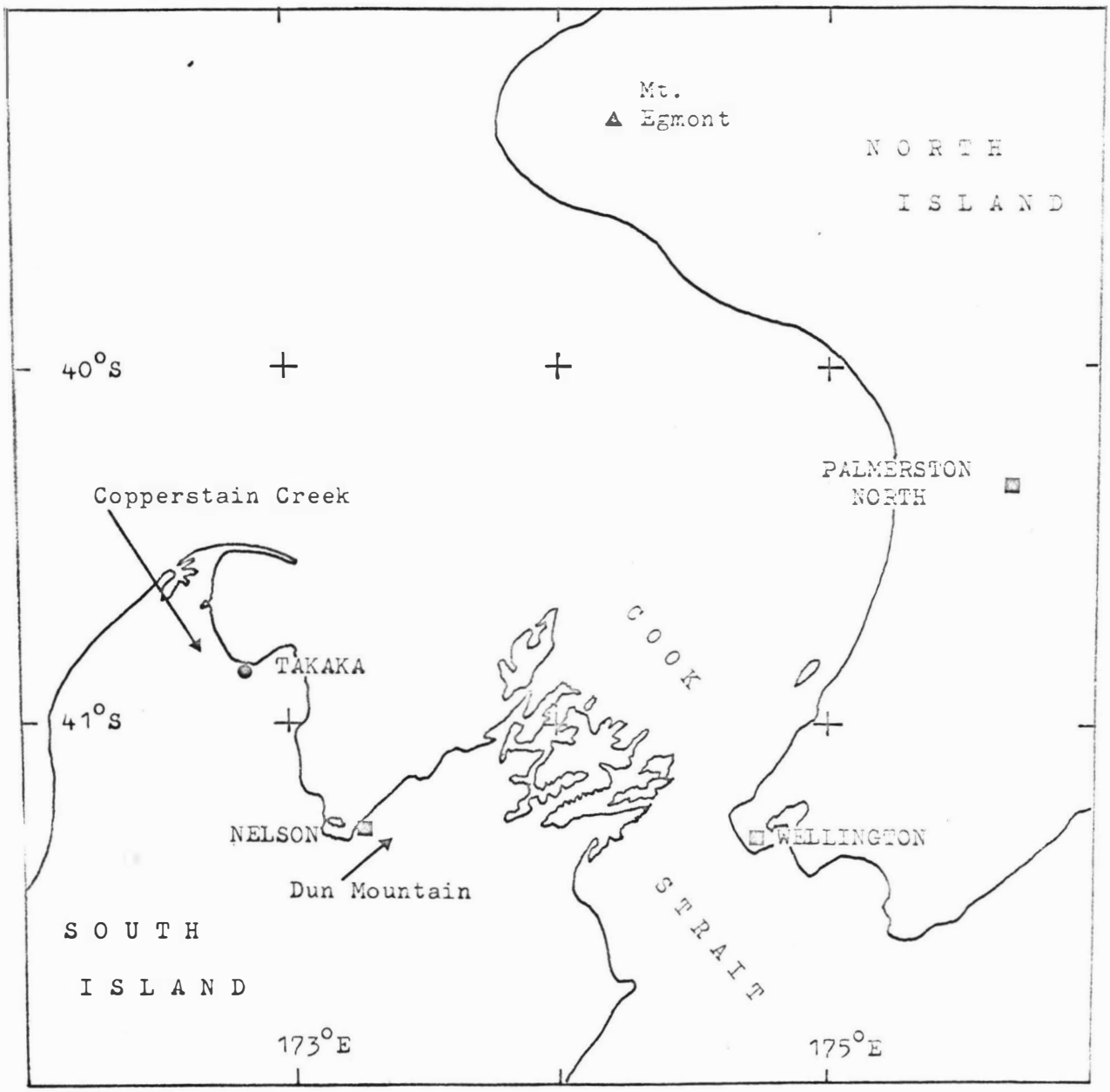

Fig. I - 1. Map of part of New Zealand showing places mentioned in this thesis.

In the present work, trace element studies of New Zealand serpentine vegetation were therefore carried out to test the biogeochemical method for chromium and copper, and to investigate the effects of serpentine soil on the composition of these plants. Serpentine soils contain unusually high concentrations of chromium, nickel, cobalt and magnesium with low amounts of calcium and other elements and it was not known what effects were produced on the elemental composition of New Zealand plants. Serpentine vegetation in other parts of the world contain very high concentrations of some trace elements (Lounamaa, 1956; Paribok and Alexeyeva-Popova, 1966) but it was thought New Zealand plants may be able to exclude some trace elements as in the case for some plants near ore deposits in Queensland (Nicolls et al, 1964-5).

The uptake and accumulation of a given metal by plants depends on a combination of factors of which soil pH, the presence of organic matter, drainage relations, the exchange capacity of the soil and the presence or absence of antagonistic elements are most important. The availability of an element in the soil is difficult to measure because, while some elements are soluble in water, others are absorbed on clay particles or bound with other parts of the exchange complex such as the humus. For any one element also, different plant species may have different mechanisms of uptake or accumulation or different requirements. Experimental work is necessary to evaluate the factors influencing mineral accumulation by plants but much can be learnt initially by the analysis of plant material and a comparison between metal content of plants and that of the soils in which they grow.

Experimental work is also necessary to elucidate the mechanisms of uptake and accumulation and determine the metabolism of many trace elements. Over ore bodies, plants may accumulate unusual amounts

of so-called "non-essential" trace elements and therefore the metabolism of these minerals may be accentuated compared with normal plants. Endemic plants may have a metabolic requirement for unusual elements.

For these reasons, investigations were also carried out on the metabolism and accumulation of unusual trace elements by radioisotope studies. Comparative work with accumulator plants and pasture plants could also be relevant to agricultural problems as other trace elements may be proved essential in the future.

The work described in this thesis concerns the first application of biogeochemical prospecting techniques in New Zealand and has been combined with studies into the plant chemistry of selected species. The purpose of these latter studies was to fulfill the dual aims of obtaining a more thorough basis for the proper understanding of biogeochemical prospecting and also to obtain useful data for the separate, though interdependent field of plant nutrition.

PART II

A BIOGEOCHEMICAL SURVEY AT COPPERSTAIN CREEK, NORTH-WEST NELSON.

1. INTRODUCTION

Copperstain Creek is a tributary of the Pariwhakaoho River about eight miles (13 km) from Takaka in North-West Nelson (Fig. I-1). Mineralisation has been known in this area for many years (Bell et al, 1907) and consists of a fifty-foot (13 m) mineralised zone, containing pyrites and altered schist, extending for about 12 chains (300 m) in a north-south direction.

Recently, this area has attracted the attention of Lime and Marble Ltd, who have carried out prospecting for sulphur and for any other associated minerals. This interest prompted the present study and it was hoped that the results of the survey, combined with geochemical stream sediment analyses and soil sampling data by Chemistry Division, D.S.I.R. (A.J. Ellis, pers. comm., 1966) would enable economic deposits to be delineated.

Stream sediment analyses by R.L. Goguel (pers. comm., 1965) had shown that copper and molybdenum appeared to be concentrated near the headwaters of Copperstain Creek and Mineral Creek, and a small lead-zinc lode had been found in Galena Creek further north. The following survey was therefore aimed at delineating copper and molybdenum mineralisation, and was later extended to include zinc data.

2. THE GEOLOGY AND GEOCHEMISTRY OF THE AREA

Intensive geological studies have been carried out by Lime and Marble Ltd (J.C. Braithwaite, pers. comms., 1965, 1966), and the New Zealand Geological Survey (A. Wodzicki, in prep.). Stream sediment and soil geochemical analyses have been made by Chemistry Division, D.S.I.R., (R.L. Goguel, pers. comm., 1965; A.J. Ellis, pers. comm., 1966). The following summaries are reproduced from their reports.

The mineralised zone that is found at Copperstain Creek occurs within a belt of Lower Paleozoic metamorphic rocks. To the east, separated by a fault, is barren and unaltered Mt Arthur marble, and Onekaka Schist lies to the west of the mineralised zone, as summarised in Fig. II-1. The mineralised zone itself contains four main rock types, amphibolites, impure marbles, granite and altered schist, but their distribution is complex. The rocks have been subjected to more than one period of faulting and have undergone varying degrees of metamorphism and metasomatism (Wodzicki, in prep.).

R.L. Goguel (pers. comm., 1965) in a geochemical survey of stream sediments from the Pariwhakaoho River and tributaries, has shown several metal anomalies. The total copper concentration ranged between 280 and 380 ppm in the minus-100 mesh fraction of sediments from the head of Mineral Creek or from high up Copperstain Creek, compared with a background of about 60 ppm. The total molybdenum content however, was generally less than one part per million, but in a tributary of Copperstain Creek, now called Moly Creek, values of 100 and 150 ppm were recorded.

Wodzicki (pers. comm., 1966) concluded that the strongest copper mineralisation occurred in impure marbles and amphibolites,

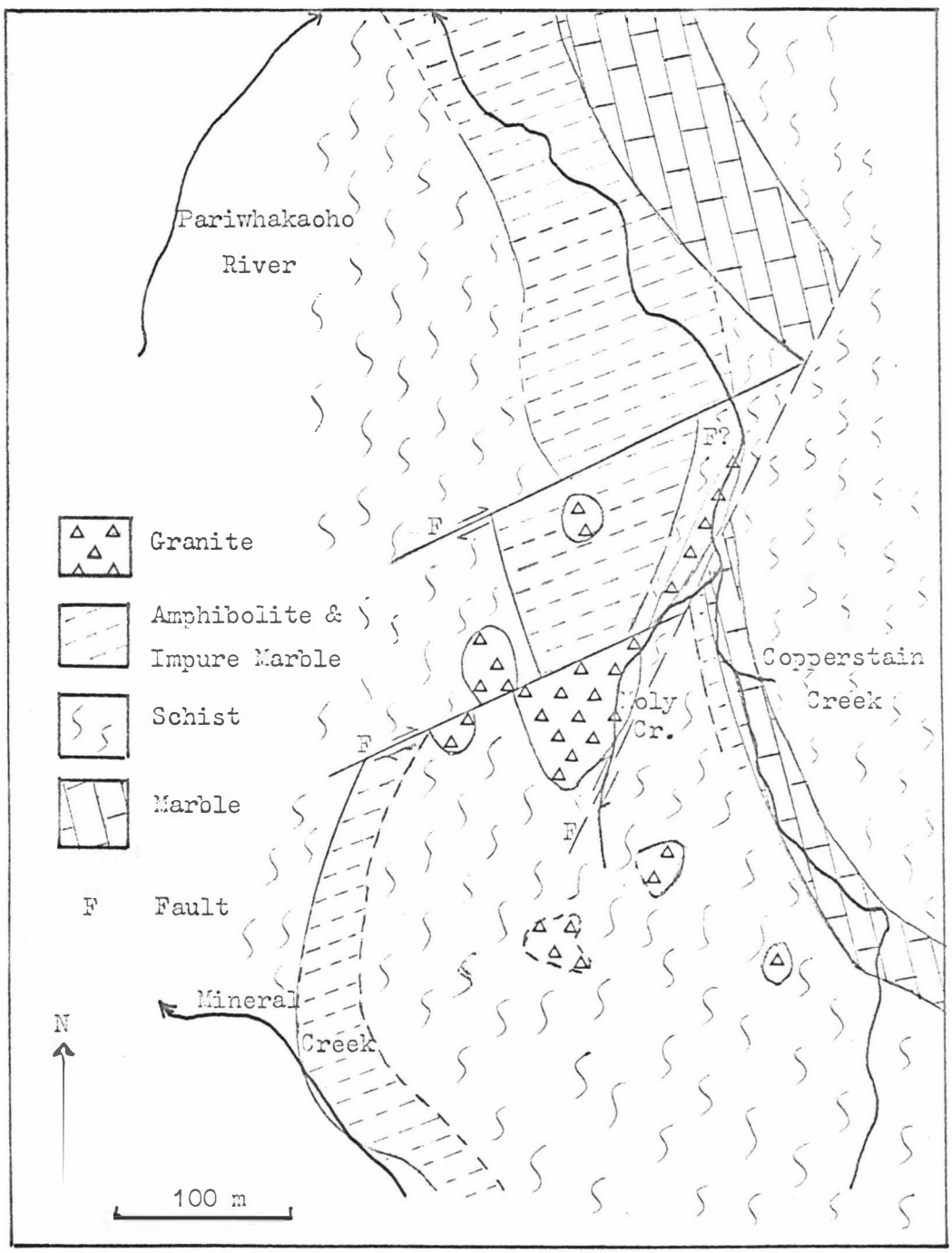

Fig. II - 1. The Geology of the Copperstain Creek Mineralised Area (after Wodzicki, in press.)

particularly those which have been moderately altered. A less intense copper mineralisation occurs in strongly-altered mica schists and feldspar porphyry. Molybdenum mineralisation appears to be related to the granite.

A soil geochemical survey was also carried out (A.J. Ellis, pers. comm., 1966). Samples were analysed by Chemistry Division, D.S.I.R., by emission spectroscopy for copper, molybdenum, lead, nickel and chromium. Contour maps showing areas of equal concentrations of these elements were made, and show that chromium concentrations were related to those of nickel, and high values are probably due to basic rocks. Lead appears to be emplaced by a separate phase of mineralisation to that responsible for copper and molybdenum anomalies. The copper and molybdenum results will be commented on further in discussion of the results.

A limited amount of diamond drilling was carried out by Lime and Marble Ltd to further delineate the extent and grade of copper, sulphur and molybdenum in the area. About 10 million tons of ore with an average content of 7.4% sulphur and 0.15% copper and a further 1.2 million tons with 0.05% molybdenum and 0.02% copper are estimated. At present prices, this ore has insufficient volume or grade for economic exploitation (P. Riley, pers. comm., 1969).

3. CLIMATE, VEGETATION AND TOPOGRAPHY

Copperstain Creek is a small tributary of the Pariwhakaoho River which flows into Golden Bay. The area drained by the creek faces north and lies less than half a mile (800 m) from where the river flows out of the hills on to alluvial land (Fig. II-2). The elevation at the mouth of Copperstain Creek is about 600 feet (200 m), rising steeply to the junction of Moly Creek at 1000 feet (300 m). The top of the ridge separating Copperstain Creek from Mineral Creek to the south-west, is at about 1600 feet (500 m).

Figure II-2 shows the steeply-dissected topography and the dense native forest of the area. The climate is generally mild but with an annual rainfall of 80-100 inches (200-250 cm) spread throughout the year except for occasional very heavy falls (de Lisle and Kerr, 1965). Erosion is rapid and the soils immature and poorly formed (A.J. Ellis, pers. comm., 1966).

The bush is of the lower beech forest zone and types characteristic of the North Island rather than the South Island are conspicuous (Bell *et al*, 1907). The canopy is dominated by red beech *Nothofagus fusca* (Hook. f.) Oerst (Fagaceae) with kamahi *Weinmannia racemosa* Linn (Cunoniaceae) common. Broadleaf species such as *Olearia rani* (A. Cunn.) Druce (Compositae), *Myrsine salicina* Hew ex Hook. f. (Myrsinaceae), and *Quintinia acutifolia* Kirk (Escalloniaceae) are common under the canopy and in clearings of regrowth after windfall.

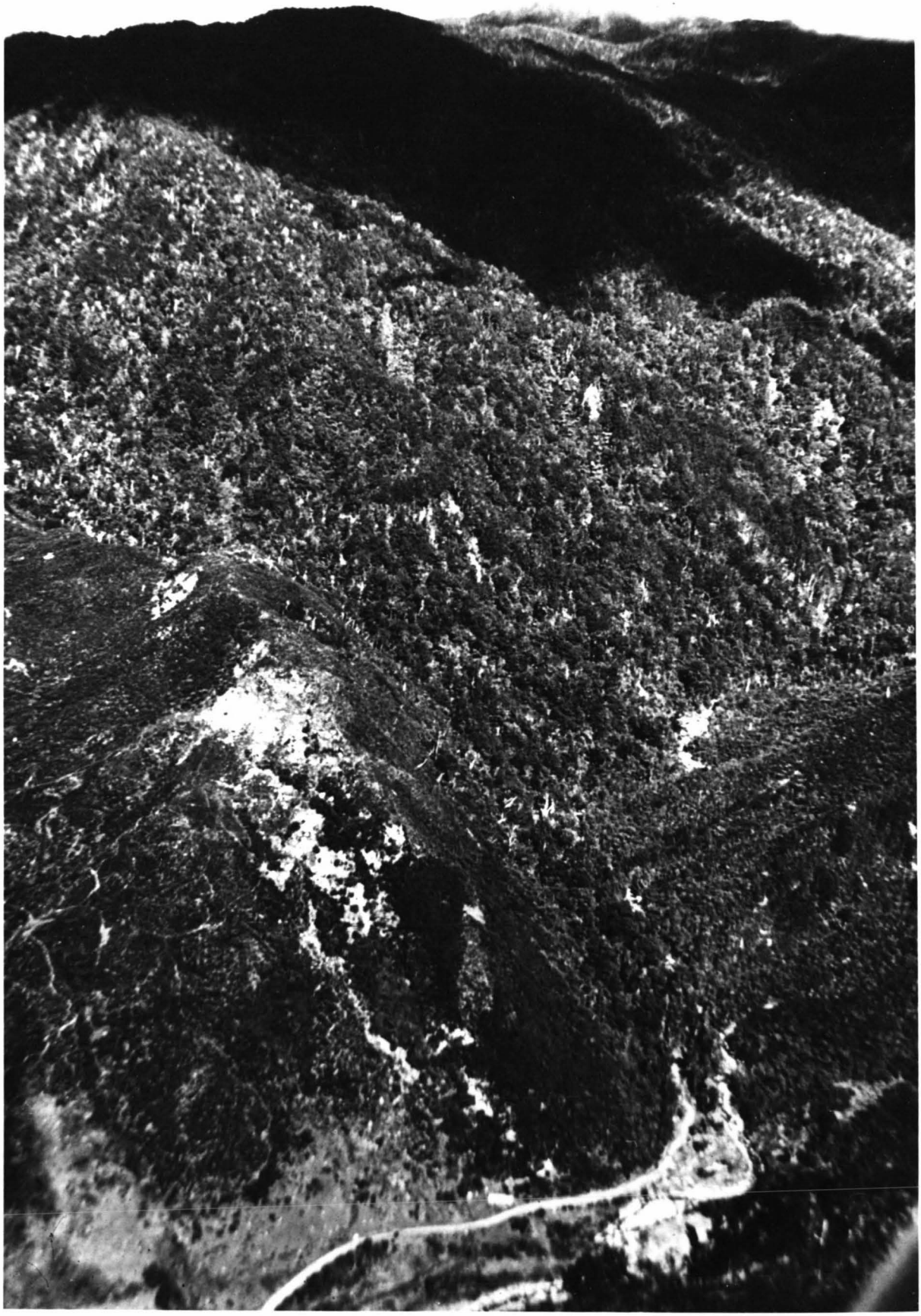

Fig. II - 2. Aerial view of Copperstain Creek

4. ANALYTICAL METHODS

(i) Plant Material

The required part of the plant was hand plucked from the tree, or removed with pruning shears. On return to the laboratory, leaves and flowers were separated from twigs and the wood was cleaned of bark. These samples were then washed quickly under fast-running tap water to remove surface dust and were dried in an oven at 110°C.

The dried plant material was then ashed in pyrex beakers in a muffle furnace at 450°C for three hours or until all the carbonaceous material had disappeared. Dry ash was used for emission spectrography but for atomic absorption analysis, 50 mg of plant ash was dissolved in 10 ml 2N hydrochloric acid and the solution was filtered and diluted as required.

(ii) Soil Samples

The material sampled was a poorly-formed soil or weathered rock underlying the humus layer. Normally soils were collected at the base of each tree specimen but for the grid survey, soils were taken at the reference point, although the trees sampled were up to 20 feet (7 m) away.

In the laboratory, soils were air dried, and sieved through a 40-mesh (1 mm) nylon sieve. Before elemental analyses, the dry soil was ignited in pyrex beakers at 450°C in a muffle furnace, and resieved through a 100 mesh (150 μ) nylon sieve, only the fines being retained. For atomic absorption analysis, 10 g of these soil samples was digested in a platinum crucible with 20 ml of a mixture of concentrated nitric, perchloric and hydrofluoric acids (4:1:5 v/v),

taken to dryness and the residue dissolved in 10 ml 2N hydrochloric acid, filtered and diluted as required.

(iii) Analysis

(a) pH

The pH of soils was measured using a standard glass electrode assembly, on the solution obtained from overnight, end-over-end shaking of about 2 g of the air-dried, sieved soil with ten times its own weight of distilled water.

(b) Emission Spectrography

Emission spectrographic analysis of plant ash and soil samples was carried out using a Hilger E 742 large automatic quartz spectrograph.

Samples were mixed with twice their weight of carbon powder which contained indium or palladium as internal standard, and were analysed in the d.c. arc under the conditions listed in Table II-1. Analytical lines used were Mo 3170, Cu 3274, Zn 3345, Pb 2833, Mn 2798, Ni 3003, V 3184, and Ag 3280. Either In 3256 or Pd 3027 was used as the internal standard line. The coefficients of variation for the concentrations of all these elements were in the range 10-15%.

(c) Atomic Absorption Spectrophotometry

Solutions of plant ash or soils were diluted with distilled water to a suitable concentration range; and standards were prepared at the same acid strength. Analysis was carried out on a Techtron AA3 atomic absorption spectrophotometer under the conditions listed in Table II-2.

Table II - 1

EMISSION SPECTROGRAPH OPERATING CONDITIONS

Wavelength range	2650 Å - 4250 Å
Slit length	12 mm
Slit width	0.015 mm
Transmission	Two-step filter. Each step 1/4.
Optical system	Convex quartz lens placed at the slit to give even illumination at this point and to provide an image of the arc at the collimator.
Electrodes	Johnson, Matthey 4B Graphite 1.6 mm internal diameter x 6 mm deep.
Arc gap	6 mm
Current	7 amps
Excitation	Anode
Atmosphere	Carbon dioxide via a modified Stallwood jet (Margoshes and Scribner, 1964)
Exposure	To completion
Photographic plates	Ilford G30
Photographic processing	4 mins at 20°C in Kodak D19 developer
Densitometry	Hilger microphotometer with Galvo-scale calibrated in B-values (Boswell and Brooks, 1965)

Table II - 2

ATOMIC ABSORPTION OPERATING CONDITIONS

	Zinc	Copper
Acetylene flow	3	3½
Air pressure	15 psi	15 psi
Flame	Reducing	Reducing
Lamp current	6 mA	4 mA
Slit width	300 μ	50 μ
Wavelength	2,139 Å	3,247 Å
Sensitivity (50% absorption)	1.8 ppm	8.5 ppm

(iv) Treatment of the Data

To be able to make careful interpretation of the plant and soil analytical results, correlation calculations were carried out on an IBM 1620 (II) computer. Programmes were written in PDQ Fortran and are recorded in Appendix 4.

Correlations between two variables are usually carried out by the method of linear regression, but Middleton (1963) notes that this is applied to determine whether one variable is dependent on the other when the latter is not subject to error. However, in this work, as in most geochemical work, neither variable can be said to be absolutely known. Therefore, it is better to calculate the reduced major axis, rather than the regression lines. The advantages of the reduced major axis are that:

1) it makes no assumptions of independence;

2) it is invariant under change of scale;

3) it is simple to compute;

and 4) results obtained from its use are intuitively more reasonable than corresponding results obtained from regression analysis (Imbrie, 1956).

For all these correlations, the logarithms of concentrations were compared, since the concentrations often spanned several orders of magnitude and appeared to be log-normally distributed (Tennant and White, 1959). Thus the position of the reduced major axis, on a log-log basis, was calculated for each pair of analytical data, and the significance of the correlation determined by calculation of the correlation coefficient (r) and reference to the tables of Fisher and Yates (1957).

In tables, the symbols used for significance are as follows:

S**	=	$P < 0.001$
S*	=	$0.001 < P < 0.01$
S	=	$0.01 < P < 0.05$
NS	=	$P > 0.05$

The geometric means and standard deviations were also calculated. The standard deviation was calculated on a logarithmic basis as for the geometric mean, and is recorded in logarithmic units. In graphs, the geometric means are marked by a cross, the limits of which show the limits of the standard deviation.

Cumulative frequency studies of some of the data were also carried out. The cumulative frequency of the concentrations of the samples, calculated as a percentage of the total number of samples, when plotted on probability paper against the concentration value, has been shown to be useful for geochemical and biogeochemical data interpretation (Tennant and White, 1959; Williams, 1967). In particular, log-normal distributions will show a straight line when plotted on log-probability paper, and similarly with normally-distributed data on linear-probability paper, otherwise curves will ensue. However, if there is more than one distribution set within the data, such as could occur with mineralised and unmineralised soil samples, a distinct change of slope or a point of inflexion in the graph will be observed. This break can be considered to occur at the minimum concentration of mineralised samples, although some overlap of distributions will occur (Williams, 1967). However, the significance of interpretation from this analysis becomes progressively more limited for decreasing numbers of samples below about one hundred.

5. PRELIMINARY SAMPLING

In a preliminary survey, four plant species, Myrsine salicina, Olearia rani, Quintinia acutifolia and Weinmannia racemosa, were sampled from seven sites in a traverse of an area, near Moly Creek, which had been shown to be mineralised by A.J. Ellis (pers. comm., 1966). These four species were selected because of their high frequency of occurrence in the area, this being a very necessary prerequisite for biogeochemical prospecting. However, not all the species were found near every site.

Leaves of the samples were analysed spectrochemically as described above, for eight elements. The results are shown in Table II-3.

For most elements analysed, leaf concentrations showed only small variations, and were not above the average contents for vascular plants (Malyuga, 1964). However, all plants contained much greater amounts of molybdenum at Site 4 than at other sites, and as there was considerable geological evidence for copper and molybdenum mineralisation, future studies were aimed primarily at delineating the mineralisation of these two elements. O. rani was unusual in that it was the only plant with detectable amounts of zinc. In view of this, and that zinc mineralisation had been found in Galena Creek 500 m north of the mouth of Copperstain Creek, zinc analyses were continued on this "accumulator" plant.

It was also found that it was very difficult to sample W. racemosa as it is a large timber tree (also known as kamahi) and foliage, in general, could not be reached without a ladder. Sampling of this species was therefore discontinued.

Table II - 3

TRACE ELEMENTS IN FOUR SPECIES OF PLANT

(ppm of ash)

Sites 1-7: sampled across a mineralised area

Site 8: background

A. Myrsine salicina B. Olearia rani

C. Quintinia acutifolia D. Weinmannia racemosa

Site	Plant	Mo	Ni	Mn	Pb	Cu	Ag	V	Zn
1	A	7	31	1500	15	64	14.0	21	<100
	B	6	21	2200	52	190	5.0	16	720
	D	2	12	1600	15	47	4.0	25	<100
2	A	34	12	1300	52	51	0.8	23	<100
	C	15	26	900	25	88	2.0	44	<100
	D	22	12	920	66	74	5.0	54	<100
3	A	49	17	3000	15	41	1.0	28	<100
	C	13	12	1100	15	44	2.0	13	<100
	D	12	17	360	20	50	8.0	18	<100
4	A	500	12	1500	15	47	0.6	20	<100
	B	230	12	560	25	110	2.0	50	<100
	C	230	21	3000	15	66	2.0	18	<100
	D	140	31	3200	31	90	2.0	25	<100
5	A	4	8	1700	20	64	0.6	25	<100
	B	17	54	6600	130	250	5.0	68	1400
6	A	4	21	3900	9	66	1.0	28	<100
	C	1	36	4300	15	60	1.0	36	<100
7	A	4	8	370	46	56	4.0	46	<100
	C	2	60	5400	31	110	4.0	46	<100
	D	4	60	8000	15	170	1.0	54	<100
8	A	6	17	4100	25	75	0.8	40	<100
	B	3	12	2500	25	110	1.0	38	220
	D	4	50	6400	25	110	0.6	46	<100

Separate analyses of leaves and twigs from the remaining three species growing near a mineralised area are given in Table II-4. Molybdenum was found in significantly greater quantities in leaves than in twigs, but the copper contents of leaves and twigs were about the same. This finding led to the policy of only sampling leaves for future analyses. A more thorough investigation (Section 7) on the trace element content of various organs of O. rani confirmed that leaf analyses were most effective for searching for molybdenum.

Table II - 4

MOLYBDENUM AND COPPER IN LEAVES AND TWIGS

(ppm of ash)

Species	Part of Plant	Mo	Cu
Myrsine salicina	Leaves	110	91
	Twigs	34	88
Olearia rani	Leaves	110	190
	Twigs	22	260
Quintinia acutifolia	Leaves	360	250
	Twigs	18	300

6. GRID SURVEY

(i) Sampling Programme

Three species of plant were sampled: Myrsine salicina, Olearia rani and Quintinia acutifolia. Sampling was carried out on an approximately 100 feet (30 m) square grid pattern, in an area bounded by Copperstain Creek in the east, the ridge to the west of Moly Creek, the junction of Moly and Copperstain Creeks in the north, and a boundary 1200 feet (350 m) south near the head of Copperstain Creek. Traverses were taken from Copperstain Creek on a compass bearing of 290°.

Not all of the three species were found at all sampling points. The frequency of occurrence at the 64 sampling sites was: 42 for M. salicina, 45 for O. rani and 44 for Q. acutifolia. If the sampling had been carried out without the restriction of a square grid, it may have been possible to find all species at suitable sampling points. Soil samples were taken at every sampling point.

Leaves and soils were analysed for copper and molybdenum using emission spectrography with indium as an internal standard. Zinc analyses for O. rani and soils were carried out by atomic absorption spectrophotometry.

(ii) Results and Discussion

The analytical results for the square grid sampling are listed in Appendix 1a, and Table II-5 lists the means and ranges. Significant results are also shown on the maps in Figures II-4 to II-7.

Table II - 5

TRACE ELEMENTS IN SOILS AND PLANTS FROM GRID SURVEY

(ppm of ash)

Material	Element	Geometric Mean	Range
Soil	Mo	72	9-215
(64 Samples)	Cu	104	32-380
	Zn	70	5-210
O. rani	Mo	13	1-260
(45 Samples)	Cu	207	25-840
	Zn	625	280-1640
M. salicina	Mo	8	1-80
(42 Samples)	Cu	86	1-210
O. acutifolia	Mo	12	1-250
(44 Samples)	Cu	142	32-380

(a) Soils

Soil analytical values were considered statistically by the method of Tennant and White (1959). Cumulative frequency plots on logarithmic-probability paper (Figure II-3) for copper, molybdenum and zinc in soils indicated that both copper and zinc values each consisted of one log-normal distribution since straight lines could be drawn through most of the points on each plot. Curves occurred when these data were plotted on linear-probability paper, indicating that the values are not normally distributed. Minor fluctuations of the slope of these frequency curves cannot be considered important because of the relatively small number of samples (64).

The molybdenum analytical values however, indicated that there are two overlapping log-normal distributions in soils, the point of intersection occurring at about 100 ppm. This change of slope probably (Tennant and White, 1959) indicates that most of the values greater than 100 ppm are due to mineralisation whereas the others are the background distribution. This then suggests that about half of the soil samples were collected from molybdenum mineralised ground.

Figures II-4 to II-6 are maps of the area with contours of equal-element concentration for molybdenum, copper and zinc in soils.

Figure II-4 shows high zinc values in soils near the northern and eastern boundaries of the area. Soils usually average about 50 ppm zinc (Hawkes and Webb, 1962) but mineralised soils (Chapman and Shacklette, 1960) may have concentrations of 100-200 ppm which are similar to the highest reported here. These high-zinc areas may be due to basic rocks as high chromium and nickel values occur

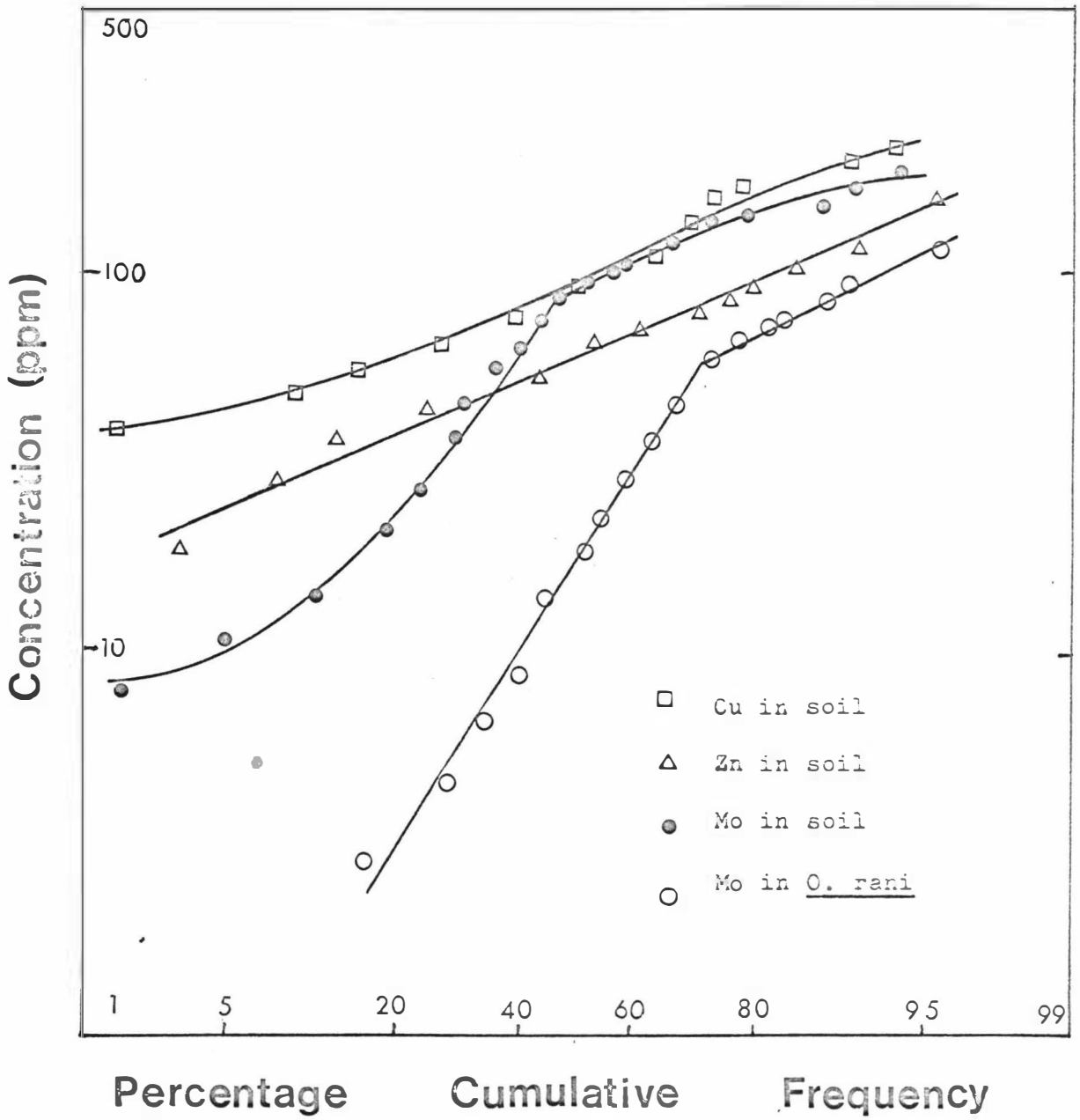

Fig. II - 3. Cumulative frecuency diagram for grid survey data.

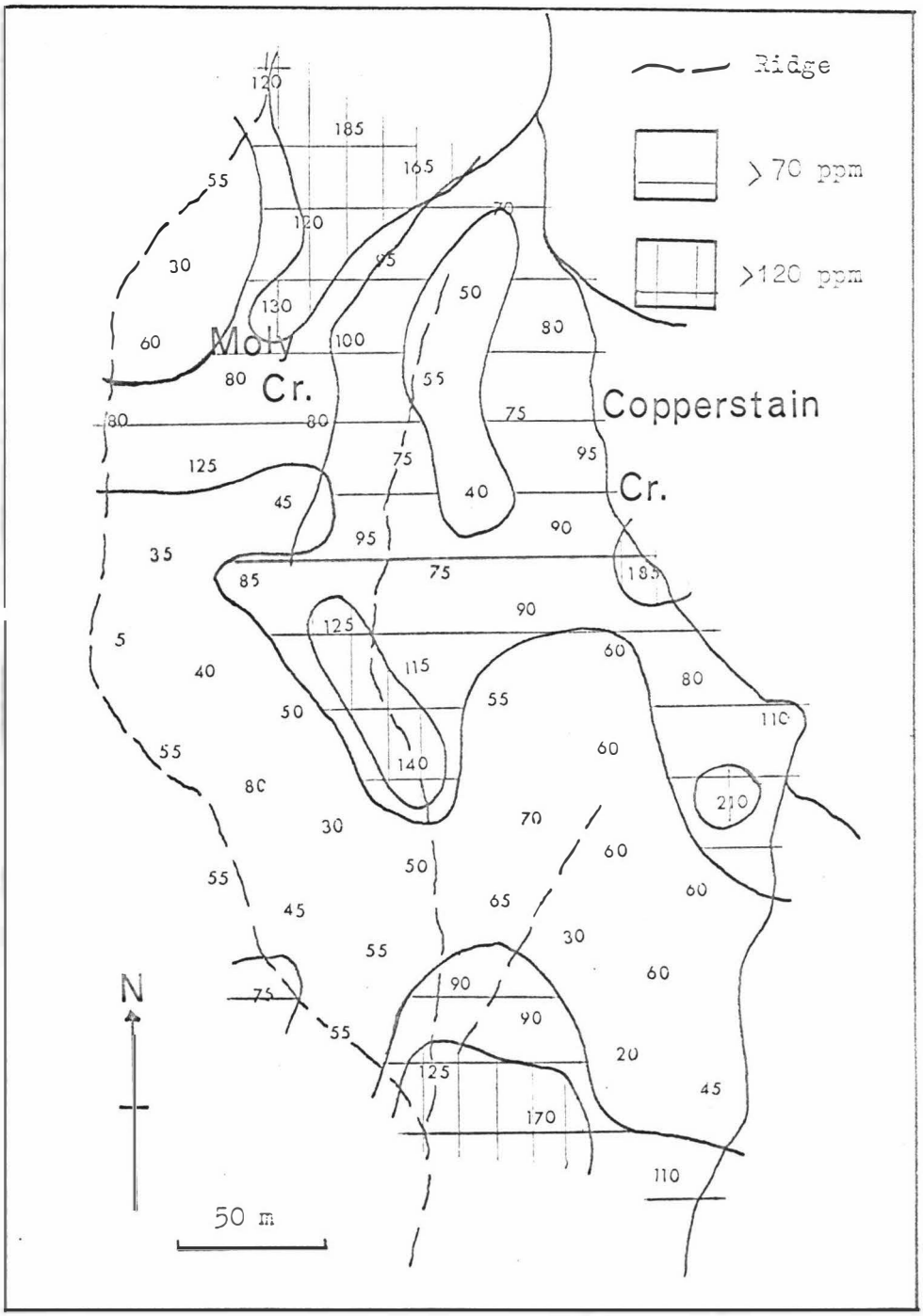

Fig. II - 4. The zinc content of soils from Copperstain Creek.

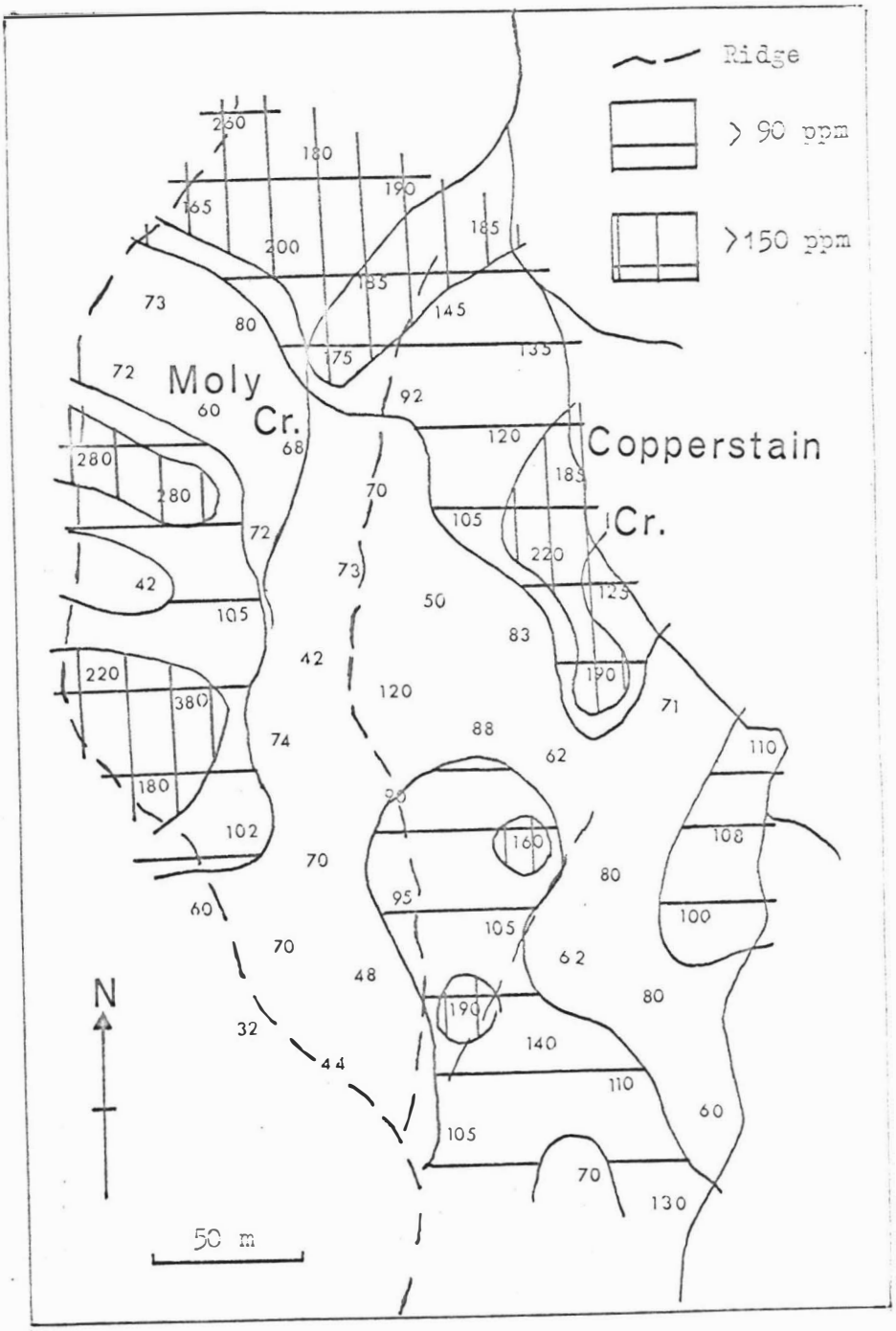

Fig. II - 5. The copper content of soils from Copperstain Creek.

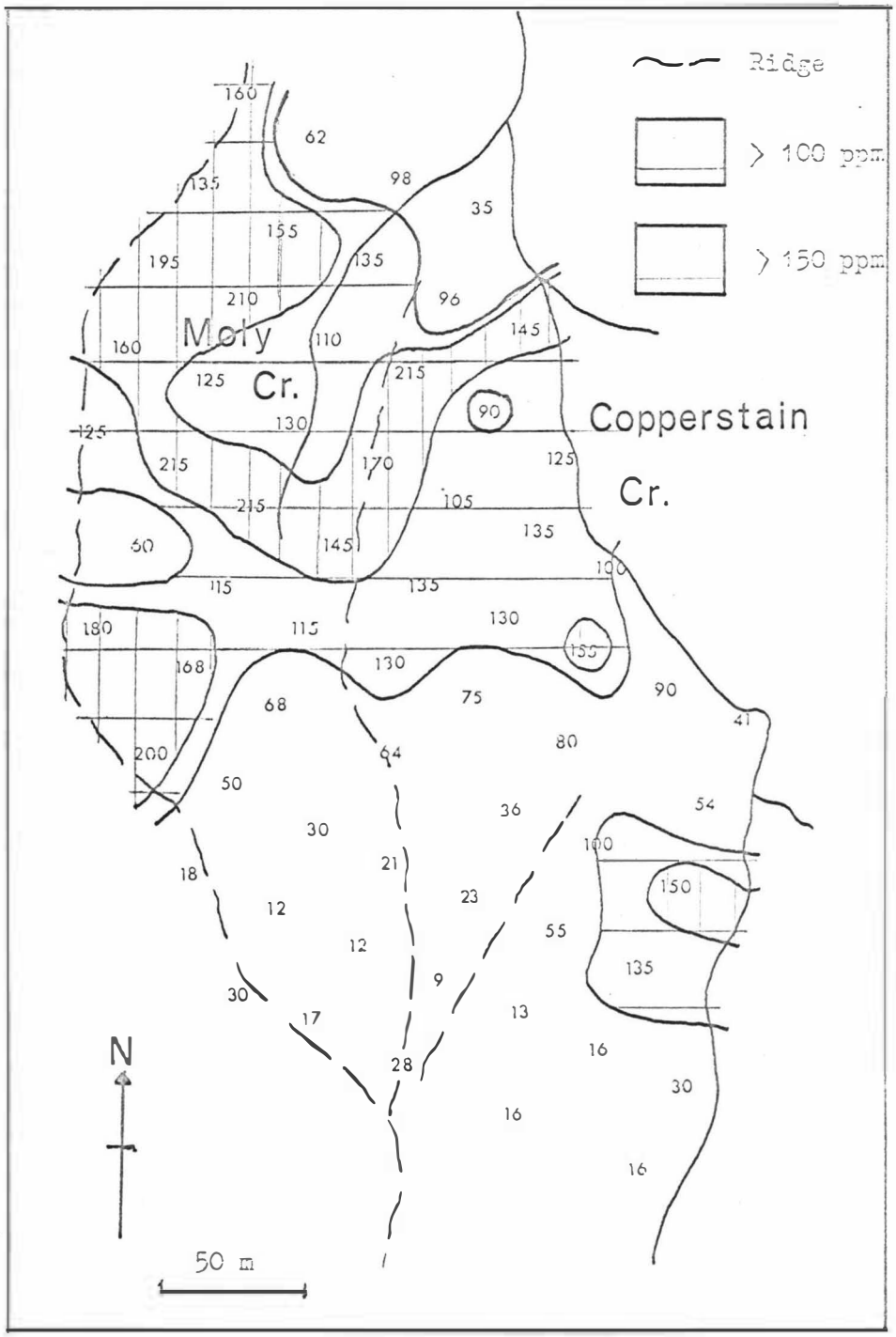

Fig. II - 6. The molybdenum content of soils from Copperstain Creek.

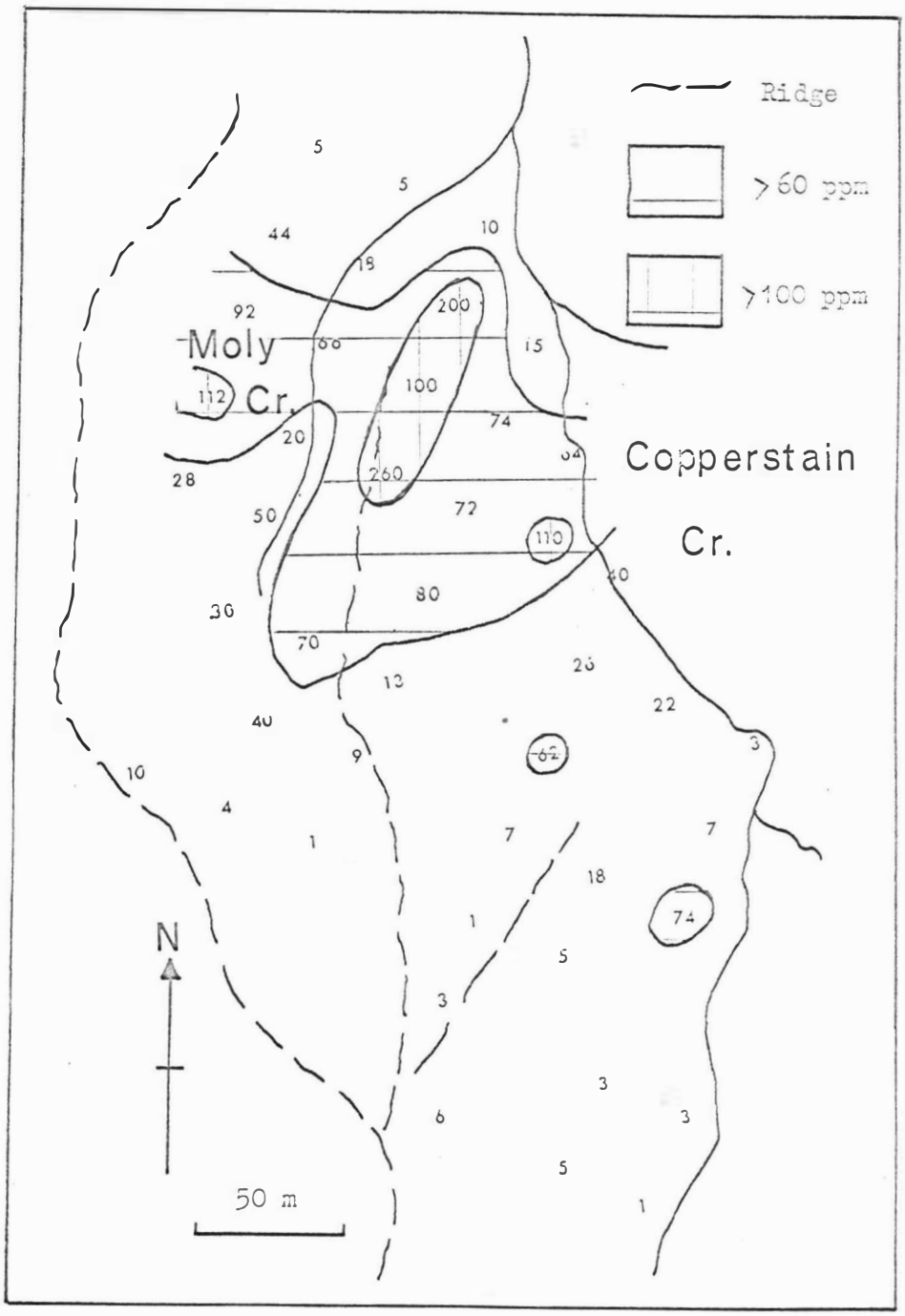

Fig. II - 7. The molybdenum content of Clearia rani plant ash from Copperstain Creek.

in the same areas (A.J. Ellis, pers. comm., 1966). However, no zinc mineralisation has been proven in this area, although a small lead-zinc lode occurs at Galena Creek 1000 m to the north.

The copper contour map for soils (Fig. II-5) shows maxima on the main ridge at the head of Moly Creek and toward the north of the area surveyed. The northern anomaly may be due in part to basic amphibolites, but elsewhere there may be copper mineralisation, especially at the head of Moly Creek. The Chemistry Division map, however, though similar to Fig. II-5, also shows a copper anomaly on the ridge between Moly Creek and Copperstain Creek. Their anomaly coincides well with that found here for molybdenum (Fig. II-6). Their molybdenum map also agrees with Fig. II-6 for the major anomalies on the ridge between Moly and Copperstain Creeks and on the main ridge to the north-west of Moly Creek.

For these contour maps, arbitrary values have been chosen for the contours, except for molybdenum where 100 ppm was taken from the cumulative frequency plot as a lower limit of mineralisation.

Table II-6 and the contour maps, show that there are no highly-significant interelemental correlations between the three elements within soils, although copper and molybdenum are slightly related ($r=+0.26$).

(b) Plants

Table II-6 shows the correlation coefficients calculated using the programme in Appendix 4d, for the concentrations of molybdenum, copper and zinc in the plants and soils. Figures II-8 to II-10 show plots of plant ash concentration against soil concentration, with the reduced major axes where there are significant correlations.

Table II - 6

CORRELATION COEFFICIENTS FOR GRID SURVEY DATA

	O. rani (45 samples)		*M. salicina* (42 samples)		*Q. acutifolia* (44 samples)		Soils (64 samples)	
Plant - Soil								
Molybdenum	+0.77	S**	+0.46	S*	+0.41	S*	-	
Copper	-0.26	NS	-0.15	NS	-0.42	S*	-	
Zinc	-0.03	NS	-		-		-	
Interelement								
Mo - Cu	+0.33	S	+0.03	NS	+0.04	NS	+0.26	S
Mo - Zn	+0.51	S**	-		-		+0.02	NS
Cu - Zn	+0.11	NS	-		-		+0.12	NS

For all three species, *M. salicina*, *O. rani* and *Q. acutifolia*, the molybdenum concentration in the plant is correlated significantly with the soil concentration. For *O. rani*, however, the correlation coefficient of +0.77 indicated a very highly significant correlation, so that *O. rani* would be a very good indicator of molybdenum mineralisation in the soil. For the other species, the lower correlation suggests that individual plant variation may disguise a mineralisation anomaly unless very large numbers of samples are taken.

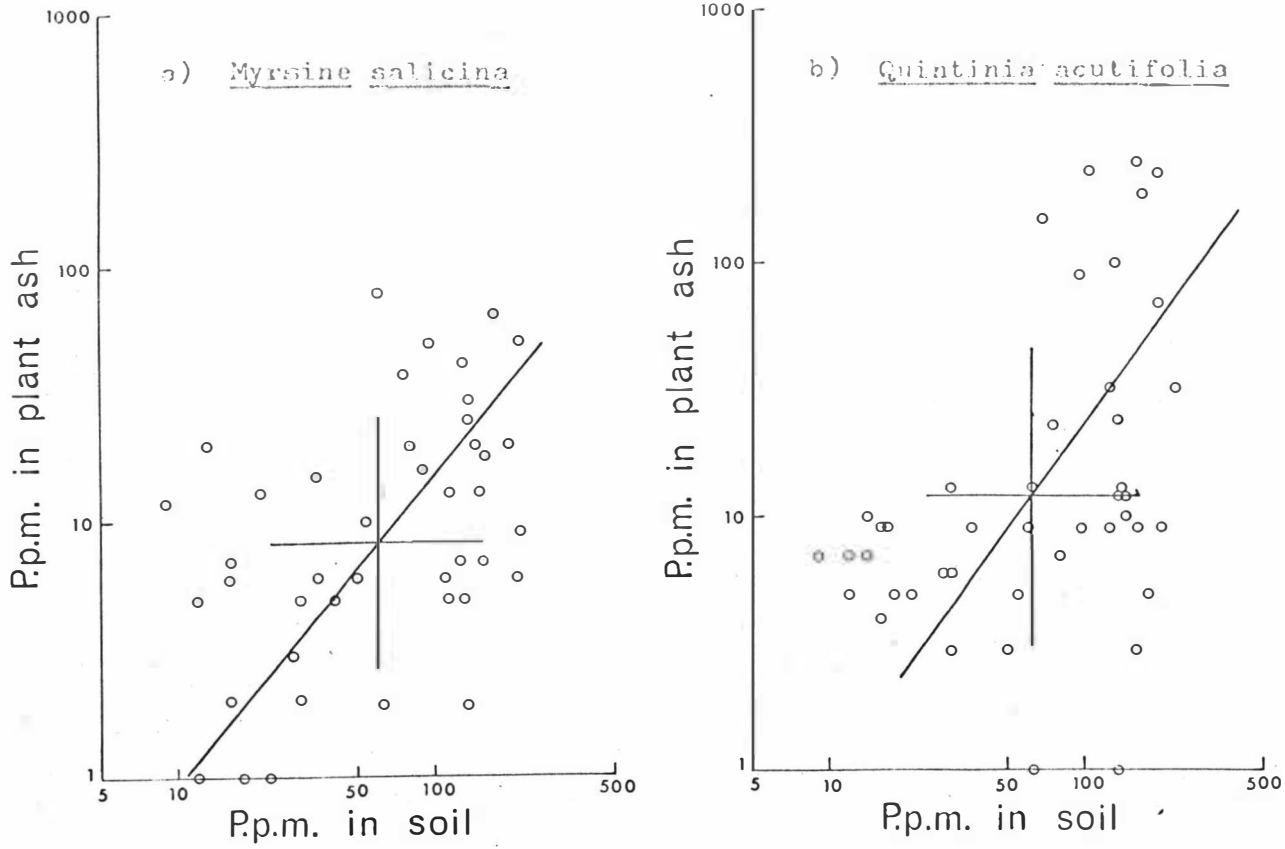

Fig. II - 8. Plant-soil relationships.
Molybdenum in plants from grid survey.

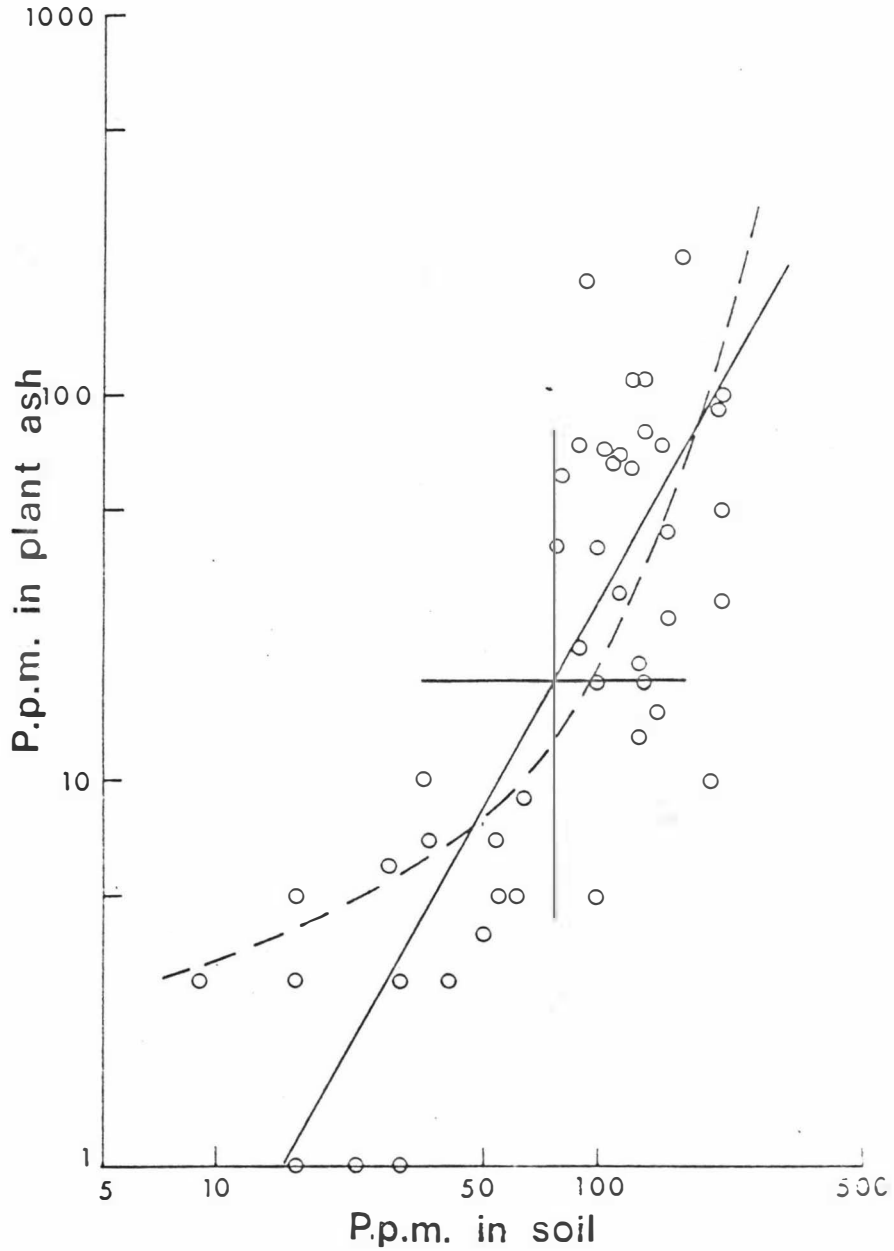

Fig. II - 9. Plant-soil relationship.
Molybdenum in Olearia rani from grid survey.

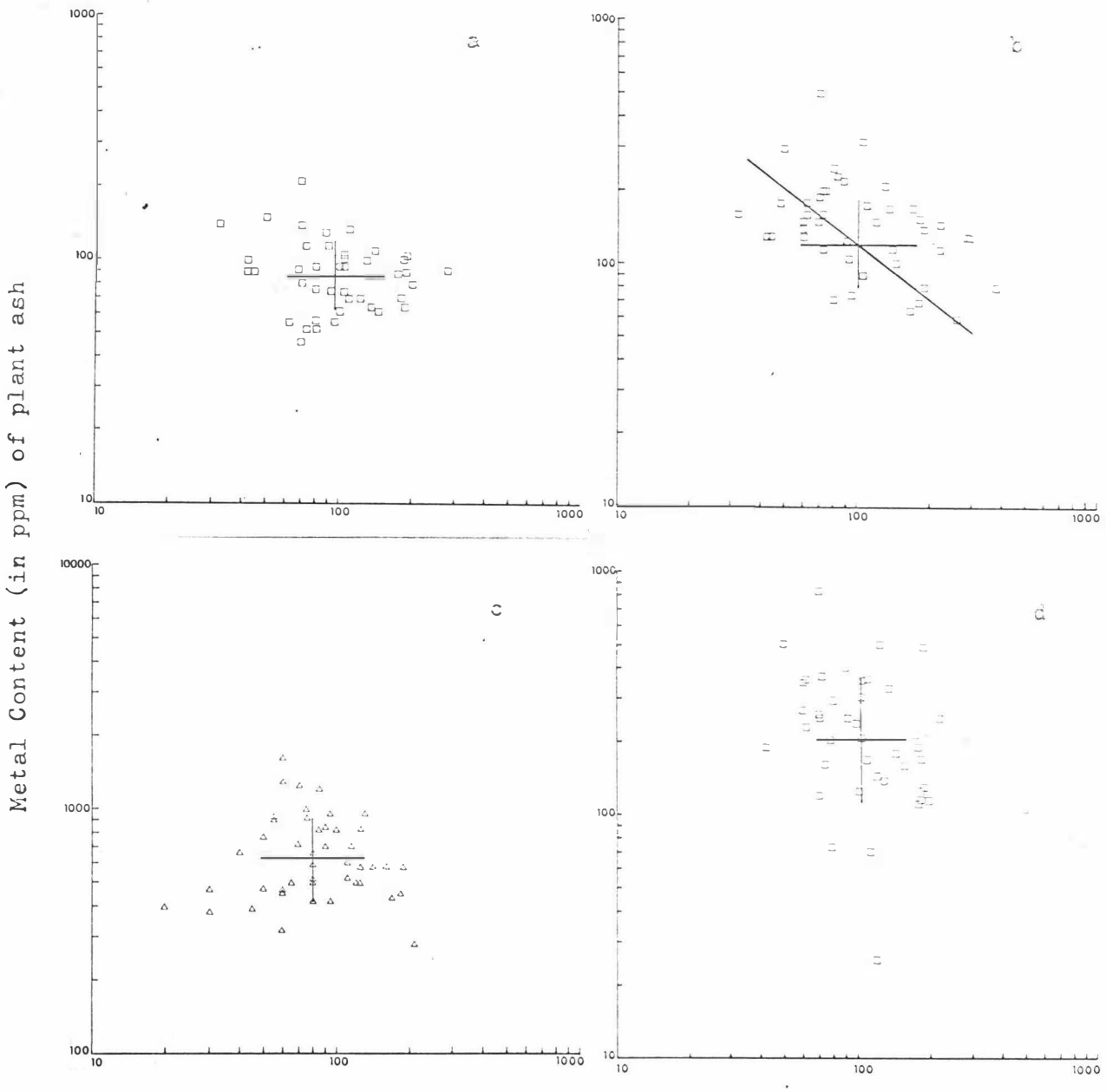

Fig. II - 10. Plant-soil relationships for plants from grid survey.
a) Copper in Myrsine salicina
b) Copper in Quintinia acutifolia
c) Zinc in Olearia rani
d) Copper in Olearia rani

The only other highly-significant plant-soil correlation is the negative correlation for copper in Q. acutifolia (r=-0.42). This is unusual, but negative biogeochemical anomalies have been observed previously (Shacklette, 1960; Nicolas and Brooks, 1969). However, the significance is unlikely to be great enough to prove useful.

When the molybdenum content of O. rani is drawn on cumulative frequency paper, (Fig. II-3), these results show two distinct straight lines, in a similar manner to the soil data, with a change of slope at a concentration of 60 ppm in the plant ash. Using this value as a threshold level, Fig. II-7 shows a map of the area with shading indicating mineralisation of molybdenum as shown by O. rani analysis. Compared with the soil contour map (Fig. II-6) the maxima on the ridge between Copperstain and Moly Creeks and to the west of the Moly Creek are similarly delineated. For the other relationships which were not as highly significant, contour maps of plant ash concentration were not in such good agreement with soil maps, and are not shown.

From Table II-5 (p. 22) also, the different contents of these elements in the three species can be noted. Molybdenum contents were, on the average, about 12 ppm in each of the species, but the mean copper contents were 207 ppm in O. rani, 142 ppm in Q. acutifolia and 86 ppm in M. salicina. It appears that these species have different requirements or different mechanisms of uptake of this element. The mean zinc content of O. rani was 625 ppm which is lower than the mean for plant ash of 1400 ppm quoted by Hawkes and Webb (1962). The only interesting interelement relationship within plants (Table II-6) is the very highly significant correlation (r=+0.51) between molybdenum and zinc in O. rani leaves.

(c) Plant-Soil Relationships

From Fig. II-9, it appeared that a curve could be drawn as the best fit through the plot of molybdenum in the ash of O. rani against soil concentration. Correlation was therefore made again but comparing the logarithm of plant ash concentration with the actual soil molybdenum content instead of the usual log-log correlation. For this log-linear relation, the correlation coefficient was +0.70, compared with +0.77 for the log-log relation, calculated using the programmes in Appendices 4a and 4b.

The reduced major axis for the log-linear relation is shown as the exponential (dashed) curve on Fig. II-9. This curve suggested that the plant may operate a partial exclusion mechanism for molybdenum, although the straight line relation with a slope of 1.8 may also imply this situation. From the exponential curve it could be seen that when the soil content was low, the plant ash content was only about one tenth that of the soil, molybdenum apparently being excluded, but when the soil molybdenum content had increased to about 100 ppm, this exclusion mechanism appeared to break down and the plant ash content increased to about that of the soil content.

A possible explanation of this would be that when the soil content is greater than 100 ppm, molybdenum occurs in a different form of mineralisation (Fig. II-3), and this form is more readily extracted by O. rani. The inference that at even higher soil concentrations this species would contain an unlimited amount of molybdenum, is of course invalid, as the plant would reach its molybdenum tolerance limit and at toxic soil levels, this species would not be found.

Nicolls *et al* (1964-5) have shown that whereas zinc in *Tephrosia sp.nov.*, and in other species in Queensland, was accumulated in proportion to the zinc soil content, the copper and lead contents indicated exclusion mechanisms. These plants had uniformly low concentrations of copper and lead over a range of 100 or 1000 fold in the soil concentration and then this exclusion mechanism appeared to break down and the plant ash concentration increased 10 or 100 times with only a further doubling of the soil concentration. These authors concluded that as the zinc content of plants bore a close relationship to the soil content, biogeochemical analysis for this element would show sharp contrast between barren and anomalous areas. This would also be the case for molybdenum in *O. rani*.

7. TRACE ELEMENTS IN OLEARIA RANI

(i) Introduction and Sampling

In the preliminary work at Copperstain Creek, it was shown that leaves and twigs of the same plant contained different amounts of the same element, and in general twigs contained less molybdenum than leaves, although about the same concentration of copper (Table II-4). At that stage in this investigation there was no opportunity to sample flowers or fruit from any of these species.

A further sampling of twenty-six specimens of O. rani was therefore made in November 1967 to collect flower heads, leaves, twigs and older wood from this species together with corresponding soils. Only O. rani was sampled as this species had proved in the grid survey to be the most useful for biogeochemical prospecting. Twigs were defined as the terminal branches just below the petioles, and wood was taken from branches 2-3 cm in diameter after removal of bark.

The purpose of this study, after the grid survey had shown that leaves of O. rani could be used for biogeochemical prospecting for molybdenum, was to provide fully comparative data for the different parts of the plant. The grid survey had been carried out completely on an ash weight basis, which had been shown by other workers (Warren et al, 1955; Malyuga, 1964) to be more useful than dry weight. Nevertheless, data for this work was calculated on an ash-weight and dry-weight basis to further test the validity of this assumption.

Zinc and copper analyses were carried out by atomic absorption spectrophotometry and molybdenum analysis by emission spectrography with palladium as internal standard.

Table II - 7

TRACE ELEMENTS IN SOIL AND IN OLEARIA RANI

(ppm of ash)

Material	Element	Geometric Mean	Range
Leaves	Mo	59	10-1600
	Cu	142	60-310
	Zn	671	340-1340
Wood	Mo	24	3-210
	Cu	231	70-480
	Zn	598	240-1780
Twigs	Mo	29	3-145
	Cu	303	60-840
	Zn	568	240-1000
Flowers	Mo	24	4-72
	Cu	206	140-360
	Zn	327	180-480
Soil	Mo	197	58-520
	Cu	180	100-410
	Zn	108	70-188

(ii) Results and Discussion

Table II-7 lists the means and the ranges for the concentrations in parts per million of zinc, copper and molybdenum in the 26 soils and in the ash of the various parts of the plants. The ash contents averaged 8.2%, 1.9%, 7.7% and 7.9% of the oven-dried weights of the leaves, wood, twigs and flowers respectively. The full data are listed in Appendix 1b.

Different parts of O. rani showed different degrees of accumulation for each element. The leaves on the average contained twice as much molybdenum as the other parts of the tree, but the flowers had only half as much zinc as the leaves, twigs and wood. Although wood ash concentrations were not much different from other parts of the plant, wood only contains about one quarter of the ash that the other organs contain. Warren et al (1955) have shown that higher average trace element contents in plant organs usually increase the reliability of those figures indicating mineralisation, and the statistical analyses shown below generally support this.

The geometric means and log-log correlations between pairs of data, were calculated from the data using the computer programme in Appendix 4c. Correlations were calculated between concentrations of the elements in all parts of the plant, on a dry-weight and ash-weight basis, and the concentration of the same element in the soil, and also between different elements in the same part of the plant. These correlations are listed in Table II-8, and show very different results for the three elements molybdenum, copper and zinc.

No highly-significant plant-soil correlations were obtained for copper, although there was a possibly significant ($r=+0.36$) relationship involving the twigs calculated on a dry-weight basis. Zinc invariably showed a negative value for the correlation coefficient

Table II - 8

CORRELATION COEFFICIENTS FOR OLEARIA RANI DATA

(26 Samples)

	Plant - Soil						Interelement					
	Mo		Cu		Zn		Mo-Cu		Mo-Zn		Cu-Zn	
Leaves												
Dry wt.	+0.48	S	+0.16	NS	-0.05	NS	-		-		-	
Ash wt.	+0.48	S	+0.08	NS	-0.14	NS	+0.37	NS	+0.40	S	+0.39	S
Wood												
Dry wt.	+0.29	NS	-0.02	NS	-0.09	NS	-		-		-	
Ash wt.	+0.29	NS	+0.03	NS	-0.30	NS	+0.34	S*	+0.42	S	+0.26	NS
Twigs												
Dry wt.	+0.45	S	+0.36	NS	-0.20	NS	-		-		-	
Ash wt.	+0.50	S*	+0.27	NS	-0.41	S	+0.15	NS	+0.39	S	+0.42	S
Flowers												
Dry wt.	+0.32	NS	+0.19	NS	-0.06	NS	-		-		-	
Ash wt.	+0.37	NS	+0.27	NS	-0.13	NS	+0.40	S	+0.44	S	+0.68	S*
Soil	-		-		-		+0.38	S	-0.28	NS	+0.17	NS

between plant and soil but this was significant only in the case of the content of twigs expressed as ppm of the ash (r=-0.41). This suggests that negative biogeochemical anomalies could be indicative of mineralisation, and indeed this has been reported in Alaska over a lead lode by Shacklette (1960) and in New Zealand by Nicolas and Brooks (1969).

For molybdenum, a number of significant correlations were apparent. The best plant-soil relationships were for leaves (r=+0.48) and twigs (r=+0.50) expressed as ppm ash weight. Neither of these values is very significant due to the small number of samples, but when these data for molybdenum in leaves are combined with the data from the grid survey, a very-highly significant correlation (r=+0.74) is obtained for the 71 samples. This significance confirms the usefulness of analysis of leaf ash to indicate molybdenum mineralisation.

However, as these 26 samples also showed significant correlations for molybdenum between soils and leaves on a dry-weight basis and soils and twigs expressed on both dry and ash weight, it is possible that these correlations would also be improved if a greater number of samples was considered.

The results show overall that elemental concentrations in the ash of the plant gave a slightly better correlation with the soil content than values expressed as ppm in dry matter. Warren et al (1955) and Malyuga (1964) have also found that analyses expressed as ash content rather than content in dry matter are generally more suitable for indicating biogeochemical anomalies.

Warren et al (1955) have discussed the advantages of analysis of different organs of the same plant concluding that "second year" twigs are the most useful indicators of copper or zinc mineralisation

in British Columbia. This work, however, suffers in that they did not analyse any soils and relied on geological and mining information for diagnosing areas of mineralisation.

When the correlations between two elements in the same plant or soil sample were considered, it was obvious that most of these interelement relationships were significant to some degree. In particular, copper showed a highly-significant correlation with zinc in the flowers and with molybdenum in the wood. Since all three elements are known to be essential to plants to some degree (Bowen, 1966), their accumulation together in the reproductive parts of the plant is not unlikely, and correlations between all three elements in flowers were significant. Molybdenum also was significantly correlated with zinc in all parts of the plant. In the soils, as found for the grid survey samples, only molybdenum and copper were significantly correlated together.

8. CONCLUSIONS

This study was the first to demonstrate that biogeochemical prospecting is possible in New Zealand. From the analyses of molybdenum and copper in the leaves of *Myrsine salicina* and *Quintinia acutifolia*, and of zinc, copper and molybdenum in leaves, twigs, flowers and wood of *Olearia rani* in the Copperstain Creek catchment, it has been shown that molybdenum mineralisation can be detected most effectively by the analysis of the leaf ash of *O. rani* for molybdenum. As with the zinc content of some plants in Queensland (Nicolls *et al*, 1964-5), the molybdenum content of *O. rani* leaf ash bore a close relationship with the soil content, and showed good contrast between anomalous and barren ground.

Thus, although the work described here was not concerned with an economic mineral deposit, it showed that analyses of *O. rani* leaves would be able to give an indication of molybdenum mineralisation. This, however, only applies to trees growing in soils of the same pH and general properties as those at Copperstain Creek. *O. rani* also has the advantage that it is very common in parts of the North Island and the northern South Island, and it can be readily recognised.

Although geological studies have shown that pyrites and chalcopyrite are present (Wodzicki, in prep.) and soil geochemical studies indicated a copper anomaly, plant analysis for copper did not indicate the same anomaly. This may be due to the small range of copper concentrations, which did not extend to the 1500 ppm that has been found above economic deposits (Malyuga, 1964). Neither soil nor plant analyses for zinc indicated its presence at economic concentrations.

This study was also one of the first to look at the trace element accumulation by New Zealand indigenous trees. A wide range of elemental concentrations was found to occur, for example from one to 1600 ppm molybdenum in the ash of O. rani leaves. This shows that single samples are insufficient to give any meaningful value to the trace element content of these species.

PART III

TRACE ELEMENTS IN A SERPENTINE FLORA

1. INTRODUCTION

Serpentine and other ultramafic soils throughout the world have been observed to support unusual flora, with the New Zealand serpentine areas being no exception. The Dun Mountain ultramafic area, near Nelson, known as the "Mineral Belt" is a zone of boulder-strewn country, often dun-coloured, almost totally devoid of trees and covered only by a sparse growth of stunted shrubs and smaller plants. The vegetation of the Mineral Belt presents a striking contrast with that of the neighbouring area, which is clothed in luxuriant beech forest (Bell et al, 1911; Betts, 1918) as can be seen in Figure III-1, an aerial photograph of the area.

Serpentine, in an ecological sense includes other ultramafic rocks such as peridotite, and accordingly, studies made of serpentine floras may include vegetation growing on peridotite as well as serpentine. Dun Mountain itself consists of peridotite but nearby are serpentine rocks, the products of hydrothermal alteration (serpentinisation) of peridotites. Chemically, serpentines are hydrated silicates of magnesium and iron.

The vegetation of serpentine areas has been intensively studied in only a few areas: e.g. Sweden (Rune, 1953), Poland (Sarosiek, 1964) and the Urals (Igoshina, 1966). No such study has been published on the New Zealand serpentine flora. However, it appears (Bell et al, 1911; Betts, 1918, 1919, 1920) that the vegetation near Dun Mountain and other New Zealand serpentine areas is similar in general aspects to serpentine

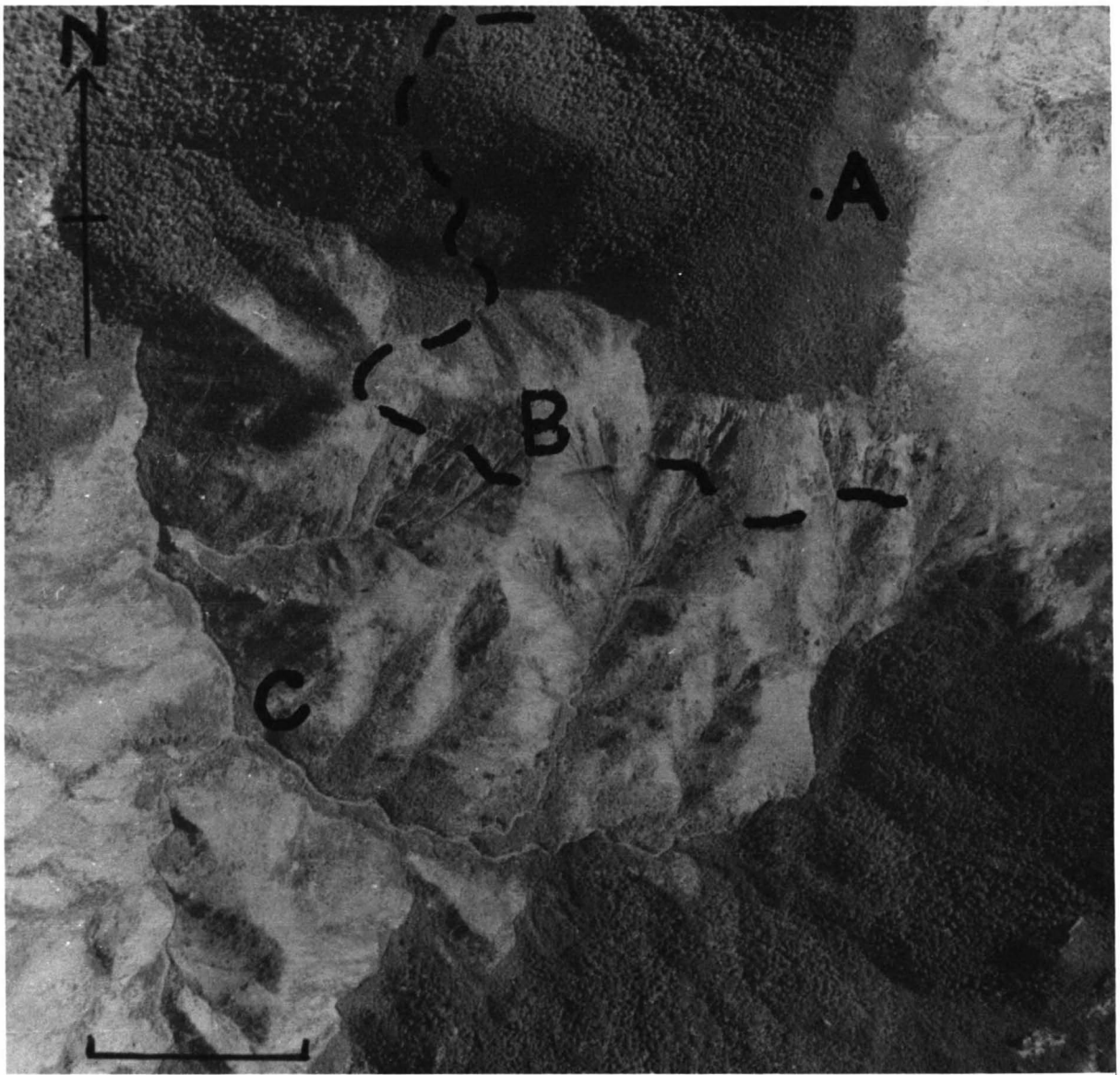

Fig. III - 1. Aerial photograph of part of the Dun Mountain Mineral Belt.

A Wooded Peak, 1110 m (3640 ft.)
B Dun Mountain Tramway
C Roding River

The scale mark is approximately 500 m

floras overseas. Thus, serpentine generally appears barren, even when below the alpine belt and surrounded by bush. On closer examination, there are often only sparse occurrences of plants and the flora is very poor in species as well as individuals. There are generally a few species or varieties which are endemic to serpentine and rare or entirely lacking elsewhere.

The cause of the unusual serpentine flora, usually attributed to the chemical composition of the soil, is not well understood, and has never been previously studied in New Zealand. Serpentine soils are considerably different to normal soils, being rich in chromium, nickel, cobalt, magnesium and iron, and low in calcium, molybdenum and the major nutrients nitrogen, potassium and phosphorus.

Walker (1954; Walker et al, 1955) and Kruckeberg (1954) considered serpentine plants to be unusually tolerant of the low available calcium content of Californian serpentine soils, which are also high in magnesium. Robinson et al (1935) made a study of serpentine soils from North America and Cuba and concluded that the excessive amount of chromium and nickel and possibly also that of cobalt is the dominant cause of the infertility of serpentine soils in which the physical conditions are favourable for plant growth. Lounamaa (1956), in a comparative study of fifteen trace elements in plants growing on silicic, calcareous and ultramafic rocks of Finland, observed that the highest chromium, nickel and cobalt values were obtained from plants growing on outcrops of ultramafic rocks. He also concluded

that the infertility and peculiar flora of the ultramafic substratum is due dominantly to the presence of chromium and nickel. Others (Rune, 1953; Minguzzi and Vergnano, 1953) considered that in particular, the excessive concentrations of nickel are responsible for the peculiarity of serpentine floras.

Recently, however, various workers in Europe and Russia (Paribok and Alexeyeva-Popova, 1966; Krause, 1958; Sarosiek, 1964) suggested that the survival of plants on serpentine soils, depends on their ability to at least partially adapt simultaneously to all the factors of a serpentine complex which are unfavourable to their development, and not only to one or some of those factors. Sarosiek (1964) concluded from a comprehensive ecological survey, that xerophytic plants are the most successful in adapting to serpentine soil, and the direct response to serpentine soil is the change in chemical composition of the plant, reflecting that in the soil. Paribok and Alexeyeva-Popova (1966) also considered that the effects of the deficiency of nitrogen, potassium, phosphorus and molybdenum and the high content of iron, are minor compared to the toxic effects of chromium, nickel and cobalt, and the unfavourable relation of magnesium to calcium with low availability of the latter.

It has been known for many years (Bell et al, 1911) that the Nelson Mineral Belt contains appreciable amounts of copper and chromium, and indeed these elements were mined in several places during the last century. In general, the deposits proved to be small and uneconomic, but the possibility remains of finding further deposits of these metals, or of others which are known to be concentrated in ultramafic rocks. The present study therefore initially

developed as an investigation into the accumulation of chromium, nickel, copper and cobalt by plants of the area in order to establish the necessary background to a future biogeochemical survey.

In order to establish not only the suitability of serpentine plants for biogeochemical prospecting, but also the range of trace element concentrations that a plant species might be able to tolerate, comparative plant and soil analyses were also made on specimens from the nearby boundary with sedimentary rocks and on some species growing in the andesitic soils of Mount Egmont.

As previous studies (Lounamaa, 1956; Paribok and Alexeyeva-Popova, 1966; Sarosiek, 1964) had shown abnormally high uptakes of nickel and chromium by plants, it was hoped that the work would also provide useful data of nutritional importance about these non-essential elements. It is known, for example, that at least in ruminants both copper and cobalt are essential (Underwood, 1962), and chromium may be also implicated in mammalian metabolism (Schroeder, 1968, Schroeder et al, 1962).

It was also hoped that the work would simultaneously provide information on the reasons for the existence of a characteristic serpentine flora. Analysis was extended to the elements calcium and magnesium to assist in this aim.

2. DESCRIPTION OF THE AREA

(i) Physical Features

Figure III-1 is a vertical aerial photograph of the area from which samples of plants were collected. The main topographic feature is the headwater of the Roding River which drains the southern slopes of Wooded Peak. The eastern slopes of Wooded Peak are drained by the South Branch of the Maitai River, while further east is the massif of Dun Mountain itself. A map of the area is shown in Figure III-2.

The line of the tramway marked on both Figures III-1 and III-2 runs north-west about 11 km (7 miles) towards the port of Nelson (Fig. I-1). Deposits of copper and chromium have been known here since the early days of New Zealand settlement and this, New Zealand's first tramway was built in the ten years from 1855. However, all the deposits in this area are small and mining has never been profitable. The tramway is still negotiable by four-wheel drive vehicle to within an hour's walk of the Mineral Belt, and provides the easiest access to it.

The area studied is of moderate relief, ranging in altitude from 730 to 950 metres (2200-2900 feet) above sea level. The climate is mild with 150-200 cm (40-50 inches) of rain spread evenly through the year (de Lisle and Kerr, 1965).

(ii) Geology

The area from which plants were collected has not been the subject of any intensive geologic study. Lauder (1965a, 1965b),

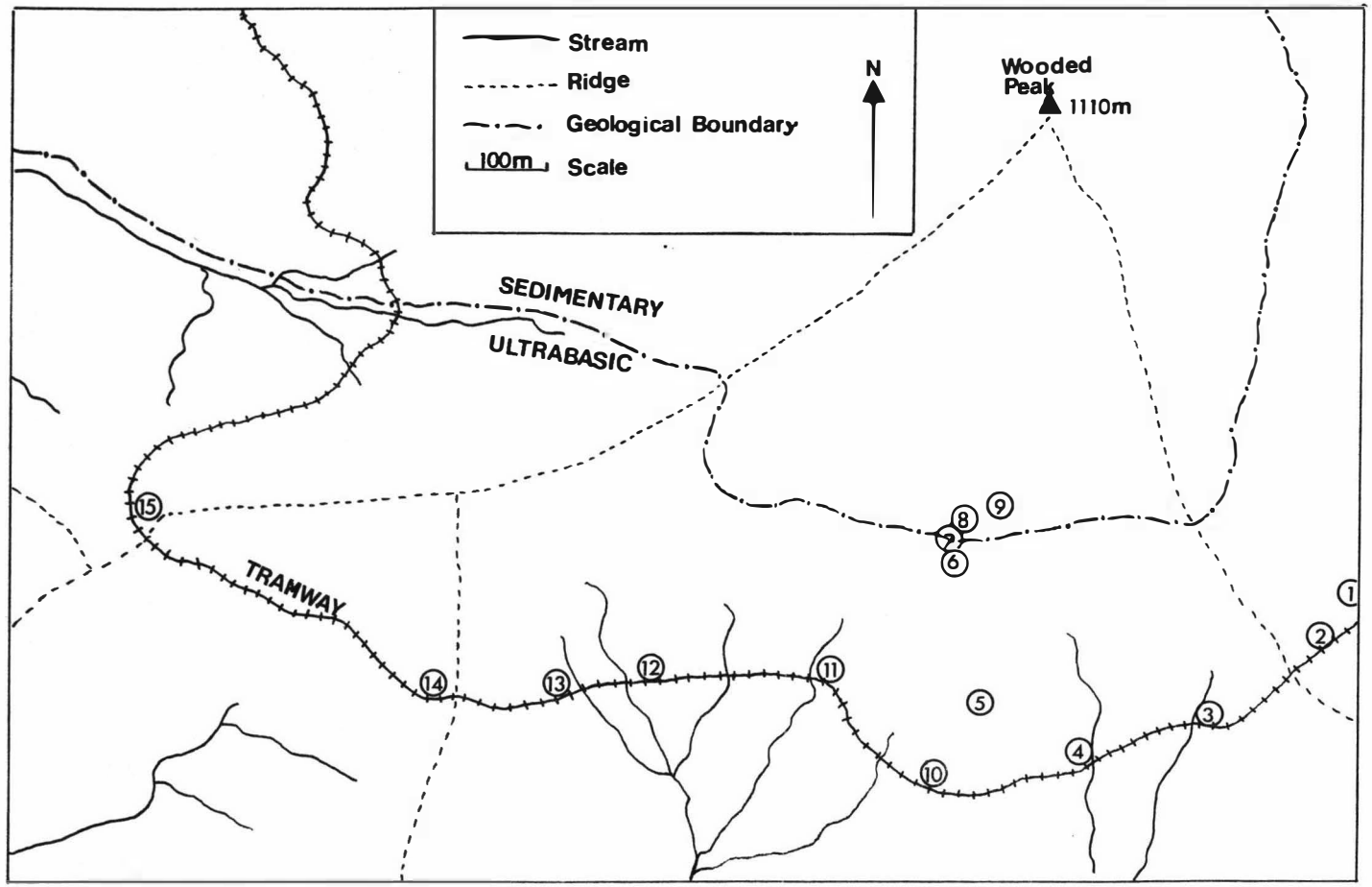

Fig. III - 2. Map of the Dun Mountain Tramway showing sampling sites.
(Note: the tramway does not extend past Site 4)

however, has made a general study of the rocks near Dun Mountain, and concentrated on the ultramafic rocks of the mountain itself. Bell et al (1911) have made observations on the igneous and economic geology of the Mineral Belt.

The underlying rocks in the vicinity of the tramway (Figures III-1 and III-2) are serpentinite with occasional lenses of rodingite. The old copper and chromium mines, of which a few drives are still visible, occur from Site 15 (Figure III-2) eastward, with most mines near and above Site 4 (Bell et al, 1911). These deposits may have been emplaced along a fault (Lauder, 1965b). Few signs of copper remain, but there are numerous boulders containing chromite and the mine dumps near the end of the tramway (Site 4) contain high concentrations of chromite-bearing material.

Plants were also collected at Sites 6 - 9 (Figure III-2) at the boundary of serpentine on the southern slopes of Wooded Peak. The Peak, and the surrounding bush-covered area (Figure III-1) is underlain by sedimentary and volcanic rocks, mainly spilite, argillite and marble of Late Paleozoic age (Lauder, 1965a). According to Waterhouse (1959), Wooded Peak is composed of the Wooded Peak Limestone, a member of the Permian Maitai Group. Near the boundary, downslope movement of these rocks broadens the vegetation boundary due to the presence of mixed soils.

(iii) Vegetation

In Figure III-1 can be seen the striking contrast between the vegetation of the Mineral Belt itself and the surrounding countryside. Figure III-3 is a closer view of a boundary with scrub and

Fig. III - 3. Photograph of head of Roding River, showing sparse vegetation on Mineral Belt and abrupt change to bush on sedimentary rock.

bare soil showing on serpentine, and heavy bush on the adjacent rocks. The rocks on both sides of the Mineral Belt support a dense beech forest of mainly Nothofagus solandri var. cliffortioides and N. fusca with occasional conifers of Libocedrus bidwillii, Dacrydium bidwillii and Phyllocladus alpinus (Bell et al, 1911).

The plant communities of the Mineral Belt are highly xerophytic with three principal associations (Betts, 1918).

(a) Shrubland

Usually found near the margin of the Mineral Belt such areas are composed of dwarfed forest species with a number of shrubs and small herbs.

(b) Open Scrubland

This association is characterised by shrubs such as Cassinia vauvilliersii and includes various herbs, some of which are endemic to the Mineral Belt.

(c) Tussock Grassland

Danthonia raoulii is the dominant species in these communities.

A small area of the Mineral Belt was studied in detail for the localised collection (see Section 5, p. 65).

3. SAMPLING AND ANALYSIS

(i) Plant Material

For samples from large plants, the terminal shoots were hand plucked or removed with pruning shears. For smaller plants and herbs, all the aerial part of the plant was taken. In the laboratory, samples were washed under fast-running water, and if necessary cleaned of visible dust contamination before drying in an oven at 110°C.

The dried plant material was then ashed in pyrex beakers in a muffle furnace at 450°C for three hours or until all the carbonaceous material had disappeared. Dry ash was used for emission spectrography, but for atomic absorption analysis, 25 mg of plant ash was dissolved in 25 ml of 2N hydrochloric acid containing 2000 ppm strontium as strontium nitrate. This solution was filtered and diluted with distilled water for analysis.

(ii) Soil Samples

The serpentine soils are poorly developed lithosols with no properly-formed horizons. Pebbles are present on the surface of the soil in most places although humus is formed from leaf-fall under some bushes. Soil samples corresponding to each plant were collected where possible from below the humus layer at the base of each plant. In some cases only one sample was taken for two or three plants with interwoven root systems.

In the laboratory, soils were air-dried, sieved through 40-mesh (1 mm) nylon, ashed at 450°C and were resieved through 100-mesh (150 μ) nylon. The air-dried, minus-1 mm soil was used for soil extractions, and the ashed fines for emission spectrography.

(iii) Analysis

(a) Emission Spectrography

Analysis of plant ash and soil samples by emission spectrography, using palladium as an internal standard, was carried out as for the Copperstain Creek samples (Table II-1, p. 14). Analytical lines used (wavelengths in Ångstroms), and their corresponding coefficients of variation for line pairs were: Pd 3027 (internal standard); Cr 2843, 17.6%; Ni 3003, 8.6%; Cu 3274, 10.5%; Co 3453, 15.0%. For concentrations of chromium greater than about 0.5%, Cr 2840 was used with a coefficient of variation of about 25%.

(b) Atomic Absorption Spectrophotometry

Solutions of plant ash, or soil extracts were diluted to suitable concentration ranges, and standard solutions prepared from analytical-grade reagents at the same acid strength. Analysis was carried out with a Techtron AA3 atomic absorption spectrophotometer under the conditions listed in Table III-1.

Table III - 1

ATOMIC ABSORPTION OPERATING CONDITIONS

	Ca	Mg	Cr	Ni	Cu	Co
Acetylene Flow (gauge)	6	5½	5	4	3½	3½
Air Pressure (p.s.i.g.)	15	15	15	15	15	15
Lamp Current (mA)	10	4	15	10	4	15
Slit Width (μ)	25	50	150	50	50	100
Wavelength (Å)	4227	2852	3579	2320	3247	2407
Sensitivity (50% absorption)(ppm)	2.8	1.4	14	8.0	7.5	35

(iv) Statistical Treatment of Data

This has been discussed fully in the previous part of this thesis (Section II-4, p. 16).

Correlation calculations were carried out between the logarithms of pairs of values using the IBM 1620(II) computer. The programmes used are recorded in Appendix 4. For significant correlations, the reduced major axis was calculated (Middleton, 1963) and used on graphs. Also the geometric means are marked by a large cross, the limits of which show the limits of the standard deviation (on a logarithmic basis).

As some plant ash samples contained chromium at a concentration less than 10 ppm, accurate measurement at this level was not possible. Also, in a few cases, after the trace element content had been measured by emission spectrography, there was not sufficient sample to enable calcium and magnesium analyses to be made. Accordingly these measurements were excluded from correlation and mean calculations.

Each table however, states the number of samples used for the relevant calculations. Where two such numbers are stated, usually the lower number is for those calculations involving plant ash chromium content, and the higher number for all others. Appendix 2 lists the actual data.

Cumulative frequency plots were also made on some sets of data (Tennant and White, 1959).

4. PRELIMINARY SPECTROCHEMICAL SURVEY

Initially it was not known what range of concentrations of trace elements would be found in New Zealand serpentine plants. This preliminary survey was therefore to determine whether plants from the Dun Mountain serpentine area accumulated trace elements, as with serpentine plants elsewhere (Lounamaa, 1956; Paribok and Alexeyeva-Popova, 1966) or if they were able to exclude elements likely to be toxic to their metabolism. Basic information on soils, and on the relative frequency of the various plant species was also required.

Thus a preliminary survey of the trace element content of 71 samples of 26 plant species was made. The plants were collected from eleven sites on the tramway track leading to Dun Mountain, and from four sites on a traverse across a boundary of the serpentine with sedimentary rocks. The sampling sites are numbered in Figure III-2.

The results of the chemical analyses are presented in Table III-2. Species identification when in doubt, was checked by Botany Division, D.S.I.R., Lincoln, and names are from Allan (1961).

(i) Soils

The greatest range of concentration encountered in the analysis of soils was for chromium which varied from 500 ppm in the bush, to 6.2% on mine tailings near the end of the tramway (Site 4), with most values about 0.5% (5000 ppm). Across the boundary of serpentine with sedimentary rocks, the concentration of chromium steadily decreased from 7600 ppm to 500 ppm (Sites 6 - 9).

Table III - 2

RESULTS OF PRELIMINARY SPECTROCHEMICAL SURVEY

(ppm of ash)

Site No.	Description of Site and Sample	Cr	Ni	Cu	Co
1	Near rodingite rock at head of South Maitai R.	-	-	-	-
	Cassinia vauvilliersii var. serpentina	370	300	100	13
	Leptospermum scoparium	210	400	125	19
	Coprosma parviflora	120	280	155	19
	Dracophyllum filifolium var. collinum	300	550	150	19
	Metrosideros umbellata	20	180	66	17
	Podocarpus totara	80	400	50	30
2	Serpentine rock at the head of South Maitai R.	2700	3500	19	100
	Lichen (species unknown)	34000	8300	130	215
2	Serpentine beyond end of tramway: soil	930	1700	20	77
	L. scoparium	1100	1650	110	70
	Myosotis monroi	3500	6100	145	230
	D. pronum	4400	1250	220	190
	Hymenanthera alpina	70	1660	120	340
	Myrsine divaricata	125	510	105	51
	Stellaria roughii	3600	1250	34	32
3	Serpentine near end of tramway: soil	5300	4600	64	280
	Pimelea suteri	3200	550	350	115
4	Old mine tailings at end of tramway: soil	62000	2400	91	126
	Cassinia vauvilliersii var. serpentina	4600	680	180	28
	Hebe odora	8500	2050	260	56
	L. scoparium	9000	1050	155	44
	Gentiana corymbifera	5400	400	120	260
	Phormium colensoi	700	380	75	23
5	Tailings below Old Horse Mine: soil	4200	3800	45	110
	H. odora	380	1200	190	54
	L. scoparium	840	900	280	50
	Myosotis monroi	2000	8000	130	330
	Notothlaspi australe	1300	2000	43	95
	Hymenanthera alpina	1200	2300	160	36
6	Above tramway near bush: soil	7600	4000	64	110
	C. vauvilliersii var. serpentina	60	260	250	39
	Coprosma parviflora	740	1100	250	60
	Nothofagus solandri var. cliffortioides	36	700	130	130
	Phyllocladus alpinus	52	460	170	60

cont.

Table III - 2 (continued)

Site No.	Description of Site and Sample	Cr	Ni	Cu	Co
7	Above tramway, in bush: soil	3800	1600	55	90
	Cassinia vauvilliersii var. serpentina	13	140	40	24
	Hebe odora	13	115	66	28
	Coprosma parviflora	44	440	80	76
	N. solandri var. cliffortioides	36	700	104	19
	P. alpinus	20	310	170	32
8	Further in bush than site 7: soil	1500	1700	70	61
	C. cunninghamii	60	140	72	25
	Dacrydium biforme	44	130	130	42
	Myrsine divaricata	52	100	150	19
	N. solandri var. cliffortioides	44	140	69	54
	P. alpinus	20	130	280	28
9	Further in bush than site 8: soil	500	1700	52	140
	C. banksii	52	96	74	36
	C. cunninghamii	52	200	88	23
	D. biforme	36	160	105	32
	N. menziesii	28	350	180	26
	P. alpinus	13	130	130	35
10	On tramway: soil	23000	5000	30	100
	H. odora	36	320	63	28
	L. scoparium	3800	4900	110	115
	Myosotis monroi	460	740	200	35
11	On tramway: soil	4900	2000	32	98
	Cassinia vauvilliersii var. serpentina	1500	1000	58	51
	H. odora	1000	1100	150	30
	L. scoparium	700	1080	90	28
	Dracophyllum uniflorum	2900	1340	110	44
	Lycopodium australianum	7700	1350	64	80
12	On tramway: soil	21000	11000	80	520
	C. vauvilliersii var. serpentina	360	2400	300	54
	H. odora	105	2100	220	42
	Leptospermum scoparium	2300	2500	170	84
	M. monroi	600	940	44	44
	D. filifolium var. collinum	300	1100	260	42
	Myrsine divaricata	580	1900	190	81
13	On tramway: soil	5000	11200	42	210
	C. vauvilliersii var. serpentina	2700	1150	170	42
	H. odora	1150	2600	250	440
	Notothlaspi australe	200	350	34	40
	Anisotome aromatica	115	540	80	28

cont.

Table III - 2 (continued)

Site No.	Description of Site and Sample	Cr	Ni	Cu	Co
14	On tramway: soil	8200	2200	52	290
	H. odora	1500	3500	210	42
	L. scoparium	4100	2100	80	48
	G. corymbifera	400	270	34	34
	Hymenanthera alpina	6000	9000	260	100
15	Tramway near Windy Point: soil	3200	2500	38	180
	C. vauvilliersii var. serpentina	2200	2000	200	155
	Hebe odora	90	740	70	120
	L. scoparium	650	900	70	155
	G. corymbifera	780	1300	270	105
	M. divaricata	850	2000	135	190
	S. roughii	350	6200	130	340

The concentrations of the other elements, nickel, copper and cobalt, were much less variable, none with a range greater than 10-fold in these samples. The copper concentrations averaged about 50 ppm, nickel 5000 ppm and cobalt 200 ppm.

In general, the soil contents of chromium, nickel and cobalt were greater than the concentrations of these elements in plant ash, but copper had a lower concentration in soil than in plant ash.

The soil pH was uniformly neutral on serpentine soils (6.9 - 7.5) decreasing to 6.6 in bush at the edge of the serpentine.

(ii) Plants

Several interesting observations can be made about the distributions of and trace element contents of the various groups of plants sampled here.

The one specimen of the lycopod, Lycopodium australianum and the one sample of lichen (unknown species) both had unusually high chromium and cobalt concentrations in their ash. These levels were greater than in most of the other plants although still less than the soil. The lichen also had a high concentration of nickel, relative to that of other species, but this plant would be much more susceptible to soil contamination. However, the one monocotyledon, a specimen of Phormium colensoi, contained lower concentrations of nickel, copper and cobalt than did most other species.

The gymnosperms Dacrydium biforme, Phyllocladus alpinus and Podocarpus totara were generally lower in chromium and nickel content than were the dicotyledons which comprised the remaining plants.

The majority of the specimens collected were dicotyledonous angiosperms and, as a group, showed quite considerable variability in trace element content. This is obvious whether comparing samples

of the same species from different areas, or different species from near the same sampling point. No other generalisations can be made from the data in Table III-2.

Lead analyses were also made on the plant samples. The plant ash contents varied from 10 to 500 ppm rather irregularly over all collection sites and plant species. These will not be considered further.

The gymnosperms (P. totara, D. biforme, Phyllocladus alpinus), the beeches (Nothofagus menziesii, N. solandri var. cliffortioides), the coprosmas (Coprosma parviflora, C. cunninghamii) and rata (Metrosideros umbellata) were all confined to only a few sampling sites, numbers 6 - 9 on the boundary of serpentine, and Site 1, by a prominent rodingite rock. Rodingite is a calcium-rich basic igneous rock and therefore different from the magnesium-rich serpentines and peridotites, and is probably a more "normal" substrate for plants, as is shown by this flora.

On the serpentine itself, the most common species were Leptospermum scoparium, which was often dominant in the neighbourhood of the tramway, Cassinia vauvilliersii var. serpentina, Hebe odora and Dracophyllum species. Less common were the other species collected, including the species endemic to serpentine, Myosotis monroi and Pimelea suteri and also Notothlaspi australe which is rare elsewhere.

(iii) Discussion

This orientation survey showed that soils in the area of study, contained a wide range of unusually high concentrations of chromium, nickel and cobalt, compared with levels in normal soils (Malyuga, 1964). However, it was realised that in some places, the soil concentration could vary by an order of magnitude over one or two

metres distance. Thus, rather than attempt to take soil samples representative of several plants, subsequent soil samples were taken at the base of each plant. For copper in the soils, despite the presence of abandoned copper mines, the copper contents were generally similar in all samples including those from the boundary areas.

The data showed that, except for copper, plant ash concentrations were generally lower than the concentrations of the same element in the soil. Also it can be seen that in areas of unusually high soil concentrations of a particular element, such as the 6% chromium on old mine tailings, the plants do not restrict their uptake of that element to a normal level. Rather, they absorb and are able to tolerate, unusually high amounts of these elements.

Forest plants, such as the gymnosperms, beeches, coprosmas and Metrosideros umbellata appear to be unable to survive on serpentine. For the beeches and some of the other species, it may be that they are unable to tolerate the xerophytic alpine environment, but the isolated Podocarpus totara and M. umbellata at Site 1 throw doubt on this suggestion. For these two species at least, it appears more likely that they do not grow on serpentine because they are unable to tolerate excesses of the elements chromium, nickel and cobalt. However, as Sarosiek (1964) and Paribok and Alexeyeva-Popova (1966) have pointed out, the mineral environment producing a serpentine flora does not only include the elements chromium, nickel and cobalt but of equal importance may be the calcium-magnesium balance in the soil, with the low contents of nitrogen, phosphorus, potassium and molybdenum also involved.

From visual observation and the above data, further sampling was restricted to six plant species. Leptospermum scoparium, Hebe odora and Cassinia vauvilliersii var. serpentina are three of the

most common species in the area studied. All are readily identified in the field, occur right to the edge of the serpentine, and also have widespread occurrences in other parts of New Zealand (although possibly as different varieties). The three serpentine-characteristic species Myosotis monroi, Notothlaspi australe and Pimelea suteri are all readily identifiable and occur in reasonable numbers in the vicinity of the Dun Mountain tramway. These endemic species were selected mainly because they might have been expected to provide evidence for their restricted distribution.

5. A STUDY OF THE ELEMENTAL CONTENT OF SELECTED SERPENTINE PLANTS

(i) Introduction

As a result of the preliminary spectrochemical survey and observations carried out during that field trip, further collection was restricted to six plant species.

All these samples were analysed for chromium, nickel, copper and cobalt by emission spectrography. Later after the acquisition of atomic absorption equipment, calcium and magnesium analyses were also made in view of the nutritional importance of these elements, especially in serpentine soils (e.g. Walker, 1954; Sarosiek, 1964).

The first part of this section concerns observations on species which are common not only in the Dun Mountain serpentine area but also elsewhere in New Zealand.

These species were:

Cassinia vauvilliersii (Homb. et Jacq.) Hook.f. var. serpentina Ckn. et Allan (Compositae), commonly called the mountain cottonwood or tauhinu;

Hebe odora (Hook.f.) Ckn. (Scrophulariaceae) also known as mountain koromiko;

and Leptospermum scoparium J.R. et G. Forst. (Myrtaceae) known as manuka.

These species are considered according to the method of choosing samples. Specimens of plants, and soil samples, were collected randomly to establish the widest range of concentrations, from a localised area to measure the minimum variation of elemental contents and from near the edge of the Mineral Belt where soils show less serpentine character. Some geobotanical observations are also presented.

Results are then given for all samples of soils and plants taken together, including the following three species which were not very common, and are confined almost entirely to the Mineral Belt:

Myosotis monroi Cheesem. (Boraginaceae);

Notothlaspi australe Hook.f. (Cruciferae);

and Pimelea suteri Kirk (Thymelaeaceae).

For all the results, only summaries and statistical information are presented here. The complete data are listed in Appendix 2.

Zinc analyses on most plants were also carried out but are not considered further. All values were within the range 100-2000 ppm with only small variation within each species, and correlations with other elements were not very significant.

(ii) Results from Three Common Species

(a) Random Collection

Thirteen samples of C. vauvilliersii, thirteen of H. odora and sixteen of L. scoparium were collected more or less at random along the line of the Dun Mountain tramway (Sites 10 - 15, Figure III-2) and beyond (Sites 1 - 4). The purpose of this study was to elaborate the findings of the preliminary survey, with more samples of each species, to determine the variability in elemental content between samples of the same species growing in soils of serpentine, and mineralised areas (mine tailings). Comparisons between species could also be obtained.

Table III-3 summarises the mean contents of the elements chromium, nickel, copper and cobalt (ppm of ash) in the plants and in their corresponding soils and also of calcium and magnesium (% of ash) in the same plants. The standard deviation (in logarithmic units) is given for each mean. The mean concentrations of copper and

Table III - 3

ANALYSIS OF RANDOM COLLECTION

(concentration in ash)

		Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)		Ca (%)	Mg (%)	Ca/Mg
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Plant	Plant
C. vauvilliersii	Mean	1300	11600	1425	2900	100	100	72	380	8.55	7.70	1.11
(13 samples)	Std. Dev.	0.43	0.38	0.25	0.14	0.46	0.23	0.30	0.17	0.20	0.22	0.37
H. odora	Mean	330	13800	1700	2900	155	135	39	365	7.48	13.1	0.57
(13 samples)	Std. Dev.	0.62	0.33	0.21	0.17	0.16	0.21	0.21	0.21	0.14	0.15	0.27
L. scoparium	Mean	2470	8950	2550	3300	105	105	95	400	13.2	9.81	1.34
(16 samples)	Std. Dev.	0.59	0.38	0.34	0.13	0.23	0.24	0.39	0.17	0.13	0.11	0.18

cobalt for soils of each species were generally similar, as were the standard deviations, but the plants showed greater variations than soils for all the trace elements.

H. odora invariably contained a much lower content of chromium (mean 330 ppm) than C. vauvilliersii (1300 ppm) with L. scoparium much higher (2470 ppm). Compared with the mean contents of the soils in which these plants grew, these differences are even more marked. H. odora was collected from soils with a mean chromium content of 13,800 ppm while the mean for L. scoparium soils was 8950 ppm. The soil chromium content for C. vauvilliersii was intermediate in value. However, all three species did occur on soils with chromium concentrations greater than 4%. Non-random sampling could easily account for either of the variations in soil or plant concentrations, but the relative uptakes of chromium from the soil, by the three species were unmistakably different. It can also be seen that H. odora had the widest chromium content variation among the samples collected, with a standard deviation of 0.62 logarithmic units.

For nickel, L. scoparium had also the highest mean ash content (2550 ppm) and a wider variation (S.D. = 0.34 log. units) than the other species. However, the copper contents of the plants and soils for all three species were relatively similar.

The cobalt contents of all soils showed only a small variation, but plant ash variations were greater. L. scoparium, as for nickel and chromium, showed the highest mean cobalt ash content (95 ppm) and the greatest variation (S.D. = 0.39 log. units) while H. odora had the lowest content (mean 39 ppm) and least variation.

Calcium and magnesium contents showed considerable differences between plant species. Since serpentine soils generally have very high magnesium levels and low calcium concentrations it was expected that the plants would reflect this feature (Robinson et al, 1935; Paribok and Alexeyeva-Popova, 1966). This was the case for H. odora with a mean ratio (by weight) of Ca/Mg = 0.57, but with L. scoparium, this was reversed (Ca/Mg = 1.34). However, L. scoparium had a relatively high mean magnesium ash content of 9.8%. C. vauvilliersii had a mean ratio of calcium:magnesium of 1.1, with both calcium and magnesium contents about 8% by weight in the ash.

Table III - 4

PLANT-SOIL CORRELATIONS FOR RANDOMLY COLLECTED SAMPLES

	Cr	Ni	Cu	Co
C. vauvilliersii (13 samples)	+0.49 NS	+0.52 S	+0.19 NS	+0.29 NS
H. odora (13 samples)	+0.73 S*	+0.16 NS	+0.13 NS	-0.26 NS
L. scoparium (16 samples)	+0.50 S	+0.33 NS	+0.18 NS	+0.42 NS

Table III-4 lists the correlation coefficients for the comparison of plant ash contents with their respective soil trace element concentrations. It shows the generally higher degree of correlation for chromium than for the other elements. The correlation coefficient for chromium in H. odora (r = +0.73), in particular shows that even with only thirteen samples, there was a high dependence of the plant ash chromium content on the total soil content of this element. There was also a significant correlation (r = +0.52) between the nickel contents of the soil and the ash of C. vauvilliersii.

Table III - 5

INTERELEMENT CORRELATIONS FOR RANDOM COLLECTION

	C. vauvilliersii (13 samples)		H. odora (13 samples)		L. scoparium (16 samples)	
Cr - Ni	-0.18	NS	+0.01	NS	+0.45	NS
Cr - Cu	-0.38	NS	+0.68	S*	+0.16	NS
Cr - Co	-0.03	NS	+0.36	NS	+0.51	S
Cr - Ca	+0.10	NS	-0.43	NS	-0.11	NS
Cr - Mg	-0.43	NS	+0.72	S*	-0.31	NS
Ni - Cu	+0.49	NS	+0.01	NS	+0.33	NS
Ni - Co	+0.61	S	-0.21	NS	+0.89	S**
Ni - Ca	-0.12	NS	-0.38	NS	-0.50	S
Ni - Mg	+0.60	S	+0.19	NS	-0.54	S
Cu - Co	+0.34	NS	+0.01	NS	+0.47	NS
Cu - Ca	-0.08	NS	-0.46	NS	-0.38	NS
Cu - Mg	+0.59	S	+0.46	NS	+0.14	NS
Co - Ca	-0.16	NS	-0.13	NS	-0.54	S
Co - Mg	+0.53	S	+0.43	NS	-0.39	NS
Ca - Mg	-0.55	S	-0.67	S*	-0.14	NS

In Table III-5, the correlation coefficients for interelement relationships in these plants are listed. Very few of these showed a high degree of significance, the highest being between nickel and cobalt in L. scoparium (r = +0.89, S**). This relationship was similar to that in the serpentine soils (see later). Another highly-significant correlation was that between chromium and copper in H. odora. These elements were not usually found to be related in other samples. Also, highly significant correlations were found in H. odora between chromium and magnesium, and (negatively) between calcium and magnesium.

The percentage of ash in the dry plant for each of six samples of each species was determined. For each plant the leaves were separated from stems, and ash contents measured separately for each part, as well as for a representative shoot (leaf plus stem) from each plant. These mean ash results are given in Table III-6. All ash contents were between 3% and 5% with little variation between species or part of the plant.

Table III - 6

ASH CONTENTS OF PLANTS

(Percentage of Dry Matter)

	Leaf	Stem	Shoot
C. vauvilliersii	4.89	4.29	4.53
H. odora	3.79	3.12	3.39
L. scoparium	3.47	3.68	3.59

Table III - 7

COMPARISON OF LEAF AND STEM ANALYSES

(ppm of ash)

Plant Species	Part of Plant	Cr	Ni	Cu	Co
C. vauvilliersii	Leaf	4000	880	95	420
	Stem	1100	2100	135	150
	Soil	28000	2400	124	310
	Leaf	1300	520	105	38
	Stem	11000	2000	200	135
	Soil	44000	2500	96	300
	Leaf	260	720	215	40
	Stem	5400	1300	240	74
	Soil	16000	2800	120	280
	Leaf	1800	740	135	28
	Stem	5200	2400	160	155
	Soil	23000	3000	125	330
H. odora	Leaf	240	520	205	19
	Stem	3400	960	285	61
	Soil	23000	3000	125	330
	Leaf	12	560	190	35
	Stem	240	480	275	42
	Soil	44000	2500	96	300
	Leaf	170	560	230	41
	Stem	270	780	370	56
	Soil	25000	2400	120	290
	Leaf	220	540	150	44
	Stem	2100	1450	580	85
	Soil	35000	1900	175	320
L. scoparium	Leaf	5000	1150	40	36
	Stem	26000	3700	190	190
	Soil	66000	1950	75	320
	Leaf	7400	1900	140	80
	Stem	14000	3700	340	195
	Soil	24000	2400	120	280
	Leaf	8000	1900	87	72
	Stem	14000	3500	180	205
	Soil	23000	3000	125	330
	Leaf	4400	700	105	44
	Stem	27000	2300	280	110
	Soil	12000	3600	145	420

Four samples of each species were separated into leaf and stem and analysed separately for the trace elements chromium, nickel, copper and cobalt, and the results are presented in Table III - 7.

Generally for chromium and cobalt, the leaf ash concentration was less than the stem ash concentration which was less than the corresponding soil concentration. This order was also the same for nickel in C. vauvilliersii and H. odora but for L. scoparium the soil content was between leaf and stem contents. For copper, leaf concentrations were again lower than stem values, but generally soil copper contents were lower again.

(b) Localised Collection

Fifteen samples of each of the three common species C. vauvilliersii, H. odora and L. scoparium were collected from the south side of a steeply-rising knoll to the east of Site 15 (Figure III-2). By considering plants from a localised area, it was hoped that plant elemental content variation within a species could be observed under relatively constant soil conditions. A small area could also be relatively well characterised ecologically.

The following ecological notes were made by Dr. P.J. Peterson, at the time of collection on January 7, 1966.

"Leptospermum scoparium was dominant (30 - 50 cm high) with some variation of habit. The more prostrate specimens in flower appeared to have larger flowers, but the size and colour was varied. Possibly co-dominant with L. scoparium were the tussocks Poa sp. and Festuca sp. which appeared robust and of good growth habit.

Conspicuous amongst this association were yellow-green plants of Phormium colensoi (Agavaceae) with an occasional tall sparse

flower head. Less conspicuous were occasional specimens of Dracophyllum filifolium var. collinum (Epacridaceae) but more commonly D. uniflorum. Both species had a conspicuous bed of humus surrounding the base of the plant, formed by their own leaf fall. D. pronum, small and with compressed habit, were common, scattered throughout the community.

Plants of Hebe odora, a few with white terminal flowers, were small and of various growth forms. Larger plants were usually not in flower but retained the old seed capsules. All plants examined retained leaves only on the terminal aspects of the branches, usually covering about one half their length. A few small plants retained leaves only on one sixth to one eighth of the branch length.

Cassinia vauvilliersii var. serpentina was not common and was not observed in flower. It was usually small in stature and often mixed with the branches of Leptospermum scoparium but inferior to it. Scattered throughout the community were occasional Gentiana corymbifera (Gentianaceae) and occasional Helichrysum bellidioides (Compositae) in flower.

Anisotome aromatica var. incisa (Umbelliferae) were common throughout, especially when near or amongst other plant cover.

Closely appressed, densely branching shrubs of Hymenanthera alpina (Violaceae) occurred occasionally within the area, with rare occurrence of Coprosma parviflora (Rubiaceae) with its less dense habit.

An occasional stunted shrub from the forest was noted e.g. Phyllocladus alpinus (Podocarpaceae) and Pseudopanax crassifolium (Araliaceae).

The soil cover was fairly open, with damp brownish shallow soil, but with some deep pockets. Scattered rocks occurred

Table III - 8

ANALYSIS OF LOCALISED COLLECTION

(concentration in ash)

		Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)		Ca (%)	Mg (%)	Ca/Mg
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Plant	Plant
C. vauvilliersii	Mean	815	9600	1330	2050	130	64	53	395	7.72	5.70	1.35
(15 samples)	Std. Dev.	0.59	0.10	0.18	0.07	0.27	0.09	0.26	0.08	0.14	0.16	0.20
H. odora	Mean	141	8500	1880	2030	163	92	30	425	8.06	13.6	0.59
(15 samples)	Std. Dev.	0.56	0.12	0.15	0.10	0.17	0.09	0.19	0.10	0.08	0.17	0.19
L. scoparium	Mean	1760	8750	1730	1930	93	67	69	390	18.7	7.31	2.56
(15 samples)	Std. Dev.	0.40	0.09	0.24	0.08	0.21	0.08	0.34	0.07	0.09	0.12	0.18

throughout, either beneath the soil or exposed with boulders of variable size up to 1 metre. A few small shingle slopes were also present."

Table III-8 lists the means and standard deviations of the samples collected near Site 15. The analytical data are in Appendix 2.

In all cases, the mean soil values from each species were similar and they had low standard deviations ranging from 0.07 to 0.12 logarithmic units. This was much smaller than those for the random sampling (0.14 - 0.38, Table III-3). The soil mean concentrations were also lower than for the random sampling for the elements chromium, nickel and copper.

The mean plant ash concentrations generally showed the same trends as those from the random sampling. In particular, the mean chromium content was lowest in H. odora and highest in L. scoparium, and the calcium : magnesium ratios showed the same order of values between species.

However, although the soils contained a much smaller range of trace element concentrations here than in the random sampling, the standard deviations for these plant ash levels were much higher than for the soils and nearly the same as for the randomly-sampled plants. Also of note is that L. scoparium, the species with the highest mean chromium content (1760 ppm), also had the highest mean calcium concentration (18.7%) while H. odora with a low chromium content had the highest magnesium concentration.

The correlations for plant-soil relationships were not significant and the coefficients are not tabulated. In all cases $+0.4 > r > -0.4$.

Table III - 9

INTERELEMENT CORRELATIONS FOR THE LOCALISED COLLECTION

	C. vauvilliersii (15 samples)		H. odora (15 samples)		L. scoparium (15 samples)	
Cr - Ni	+0.86	S**	+0.06	NS	+0.56	S
Cr - Cu	+0.28	NS	-0.26	NS	-0.15	NS
Cr - Co	+0.82	S**	+0.40	NS	+0.67	S*
Cr - Ca	-0.37	NS	-0.15	NS	-0.26	NS
Cr - Mg	+0.46	NS	-0.06	NS	-0.19	NS
Ni - Cu	+0.52	S	+0.43	NS	+0.18	NS
Ni - Co	+0.89	S**	+0.66	S*	+0.83	S**
Ni - Ca	-0.50	S	-0.16	NS	-0.45	NS
Ni - Mg	+0.44	NS	+0.11	NS	-0.07	NS
Cu - Co	+0.34	NS	+0.02	NS	+0.35	NS
Cu - Ca	-0.09	NS	-0.48	NS	+0.32	NS
Cu - Mg	+0.01	NS	-0.32	NS	-0.17	NS
Co - Ca	-0.56	S	-0.30	NS	-0.40	NS
Co - Mg	+0.43	NS	+0.33	NS	-0.13	NS
Ca - Mg	+0.10	NS	-0.01	NS	-0.45	NS

Table III-9 shows the correlation coefficients for interelement relations in the localised sampling. There are highly-significant correlations between cobalt and nickel in all three species, and C. vauvilliersii also has very highly significant relationships between chromium and nickel and between chromium and cobalt. The three elements chromium, cobalt and nickel are related transition metals and occur chiefly in ultramafic rocks.

(c) Non-Serpentine Samples

Apart from the specimens considered above, a few samples of the three species, C. vauvilliersii, H. odora and L. scoparium were collected from near the boundary of the serpentine near Wooded Peak (Sites 6 - 9, Figure III-2). Due to downslope movement, the soils were probably mixed serpentine and non-serpentine.

The mean elemental contents of these samples are listed in Table III-10. In all samples, the contents of chromium, nickel, cobalt and magnesium were lower, and that of calcium higher than for most of the samples growing on the serpentine. The mean calcium : magnesium ratios of 3.9 to 6.5 were very much greater than for any of the serpentine samples. Also, a number of samples contained undetectable amounts (< 10 ppm) of chromium. Mean values for chromium in plant ash are therefore misleading, being only for the lower number of samples listed, which were those with measurable amounts of chromium.

In August 1966 a number of the same common New Zealand species were collected from near Dawson Falls, Mount Egmont, North Island. Mount Egmont is an apparently extinct andesitic volcano, and Dawson Falls has a similar climate and altitude to that of the Dun Mountain tramway area. Although the species collected are the same as from

Table III - 10

ANALYSIS OF BOUNDARY COLLECTION

(concentration in ash)

		Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)		Ca (%)	Mg (%)	Ca/Mg
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Plant	Plant
C. vauvilliersii	Mean	13	2140	225	1200	86	83	13	158	10.6	1.66	6.41
(6-10 samples)	Std. Dev.	0.48	0.21	0.26	0.08	0.20	0.11	0.40	0.10	0.08	0.21	0.22
H. odora	Mean	18	1850	440	1200	68	89	17	143	12.9	3.25	3.97
(3-10 samples)	Std. Dev.	0.14	0.21	0.10	0.09	0.14	0.13	0.40	0.12	0.08	0.09	0.15
L. scoparium	Mean	220	2740	430	1160	77	74	22	163	16.9	4.16	4.07
(5-6 samples)	Std. Dev.	0.33	0.19	0.38	0.11	0.23	0.06	0.32	0.15	0.05	0.10	0.11

Table III - 11

ANALYSIS OF MT. EGMONT COLLECTION

(concentration in ash)

	Sample No	Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)		Ca(%)	Mg(%)	Ca/Mg
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Plant	Plant
C. vauvilliersii	EC1	<30	32	20	<10	110	100	10	24	8.5	5.1	1.67
	EC2	<30	25	<20	<10	66	90	12	20	12.0	5.0	2.40
	EC3	<30	40	<20	<10	46	45	8	26	13.0	4.9	2.70
	EC4	<30	30	<20	<10	80	140	8	19	14.0	5.7	2.50
H. odora	EH1	<30	30	45	<10	90	100	13	20	9.0	6.2	1.45
	EH2	<30	30	15	<10	100	80	7	19	13.5	7.7	1.75
	EH3	<30	32	30	<10	92	100	9	24	7.8	7.3	1.07
	EH4	<30	25	25	<10	70	90	7	20	12.5	8.2	1.53
L. scoparium	EL1	<30	22	15	<10	69	76	9	14	17.0	5.6	3.05
	EL3	<10	35	<20	<10	53	170	7	11	16.5	6.5	2.50

serpentine, the plants may be different varieties.

The results of the analyses of these ten plants are given in Table III-11, together with analyses of their corresponding soils. Two other samples of Leptospermum were collected and had similar trace element contents, but were L. ericoides.

Soil concentrations of chromium, nickel and cobalt at Mount Egmont are all much lower than those for serpentine soils (e.g. Table III-8), and also lower than in the boundary soils (Table III-10). However, copper concentrations are about 80-100 ppm in plants and soils from all collections.

The chromium content was below detection limits in all the Mount Egmont samples and nickel was undetectable in some of them.

All the samples from Mount Egmont had calcium : magnesium ratios greater than unity, although lower than the Mineral Belt boundary samples. This could be because the adapted serpentine ecotypes may be able to exploit the relatively luxurious boundary environment better than can normal plants (cf. Walker et al, 1955).

(d) Some Geobotanical Observations

During the collection of the random samples, a large number of L. scoparium (manuka) flowers were observed to have deeply-coloured pink centres and in some cases pink or variegated petals. It was considered that the colouration of these flowers might be dependent on the trace element content of the soil. This phenomenon has been noted overseas (Malyuga, 1964; Shacklette, 1964). However, samples collected by Dr. M.E.U. Taylor in March 1966, showed that there was apparently no correlation between the intensity of colour and any of the trace element contents of chromium, nickel, copper or cobalt in the plant shoots or their corresponding soils.

Recently, W.S. Ting (pers. comm., 1967) has shown that abnormal nickel contents of pine trees may induce alterations in the shape of their pollen grains. This effect was tested on samples from the Nelson Mineral Belt. Samples of flowers, as well as shoots and soils, were collected in February 1967. Only C. vauvilliersii and L. scoparium were flowering at the time. The flowers containing pollen were sent to Dr. William S. Ting for study.

Table III-12 lists the results of trace element analysis of the plants and soils and the mean pollen grain size for each plant. Thirty pollen grains from each plant were measured for L. scoparium and fifty from each for C. vauvilliersii. Rank coefficient calculations were carried out by Dr. Ting and are quoted in the table. These are relatively simple to calculate (Kendall, 1962) and have the advantage that a normal distribution is not assumed.

For these small numbers of samples, the relatively low rank coefficients indicate no rank correlation was better than significant at the 5% level of probability. However, it may be noticed that, for both species, the highest rank correlations were between pollen size and the plant ash copper content.

Table III - 12

RESULTS OF POLLEN ANALYSIS

	Sample No.	Mean Grain Size (μ)	Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)	
			Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil
L. scoparium	L1	16.65	75	13000	440	6400	41	110	8	480
	L2	18.02	1150	90000	420	2700	66	135	18	360
	L3	17.36	7200	25000	1900	3800	26	90	130	380
	L4	15.17	4100	10500	860	3100	48	125	56	330
	L5	14.74	850	5000	430	4700	46	180	24	460
	L6	14.14	290	12000	380	6000	32	120	15	700
	L7	16.67	65	5400	200	2300	54	110	13	340
	L8	16.65	48	3700	100	1300	26	85	10	140
	L9	16.07	620	6200	1700	3400	50	78	62	310
	L10	16.70	380	10500	630	1800	27	150	15	440
	L11	17.63	680	4400	740	4100	53	85	42	480
Rank coefficient			+0.16	+0.29	+0.18	-0.26	+0.30	-0.22	+0.05	+0.09
C. vauvilliersii	C1	24.25	7200	90000	420	2700	55	135	30	360
	C2	23.90	130	23000	400	5600	32	200	11	710
	C3	24.75	4100	20000	700	2500	99	105	32	270
	C4	25.07	<15	1600	250	1400	91	340	15	140
	C5	22.65	<15	900	140	710	53	96	10	83
	C6	21.65	540	2000	830	3800	45	140	66	390
Rank coefficient			+0.16	+0.45	-0.23	-0.28	+0.79	+0.31	0	-0.17

(iii) Overall Results

(a) Soils

One hundred and eighty-eight soil samples from the above collections, were considered together. In Table III-13, the means and standard deviations for these samples are listed with the means and standard deviations for the forty localised soil samples.

Table III - 13

SOIL ANALYSES
(ppm of ash)

		Cr	Ni	Cu	Co
Localised Collection (40 samples)	Mean	8930	2000	73	405
	Std. Dev.	0.10	0.09	0.09	0.08
Total Collection (188 samples)	Mean	7480	2540	102	330
	Std. Dev.	0.36	0.20	0.21	0.21

For all four trace elements, the standard deviations (and ranges) for the whole group of samples were at least twice those for the soils from the localised sampling. The means of the two sets differed due to the inclusion of mineralised and boundary samples in the complete group.

Cumulative frequency calculations on the soil data were made (Tennant and White, 1959). When plotted on logarithmic-probability paper, the data for all four elements showed straight line graphs. This implied that the distributions of each of the elements chromium, nickel, copper and cobalt are log-normal, and that there was only one distribution for each element.

Table III - 14

INTERELEMENT CORRELATIONS FOR ALL SOILS

(188 samples)

	Cr-Ni	Cr-Cu	Cr-Co	Ni-Cu	Ni-Co	Cu-Co
Correlation Coefficient	0.39	0.19	0.45	0.49	0.69	0.26
Significance	S**	S*	S**	S**	S**	S**

In Table III-14 are listed the correlation coefficients for various pairs of elements in the soils. The most significant correlation is between nickel and cobalt and the data are also shown in Figure III-4, together with a plot of chromium against copper. Chromium, nickel and cobalt in particular are geochemically related and from these data, it is apparent that all three concentrations increase together in these serpentine soils. In the graphs, the ranges, means and standard deviations of all four elements are also shown.

The total elemental content of soils as determined by emission spectrography is however not a true measure of the amount of the element available to the plants (Mitchell 1957, 1964). Twenty four soil samples from various sites near the Dun Mountain tramway were therefore extracted with 2.5% acetic acid solution. This extractant is not the perfect indicator for availability of plant nutrients, but is commonly used (Mitchell, 1964).

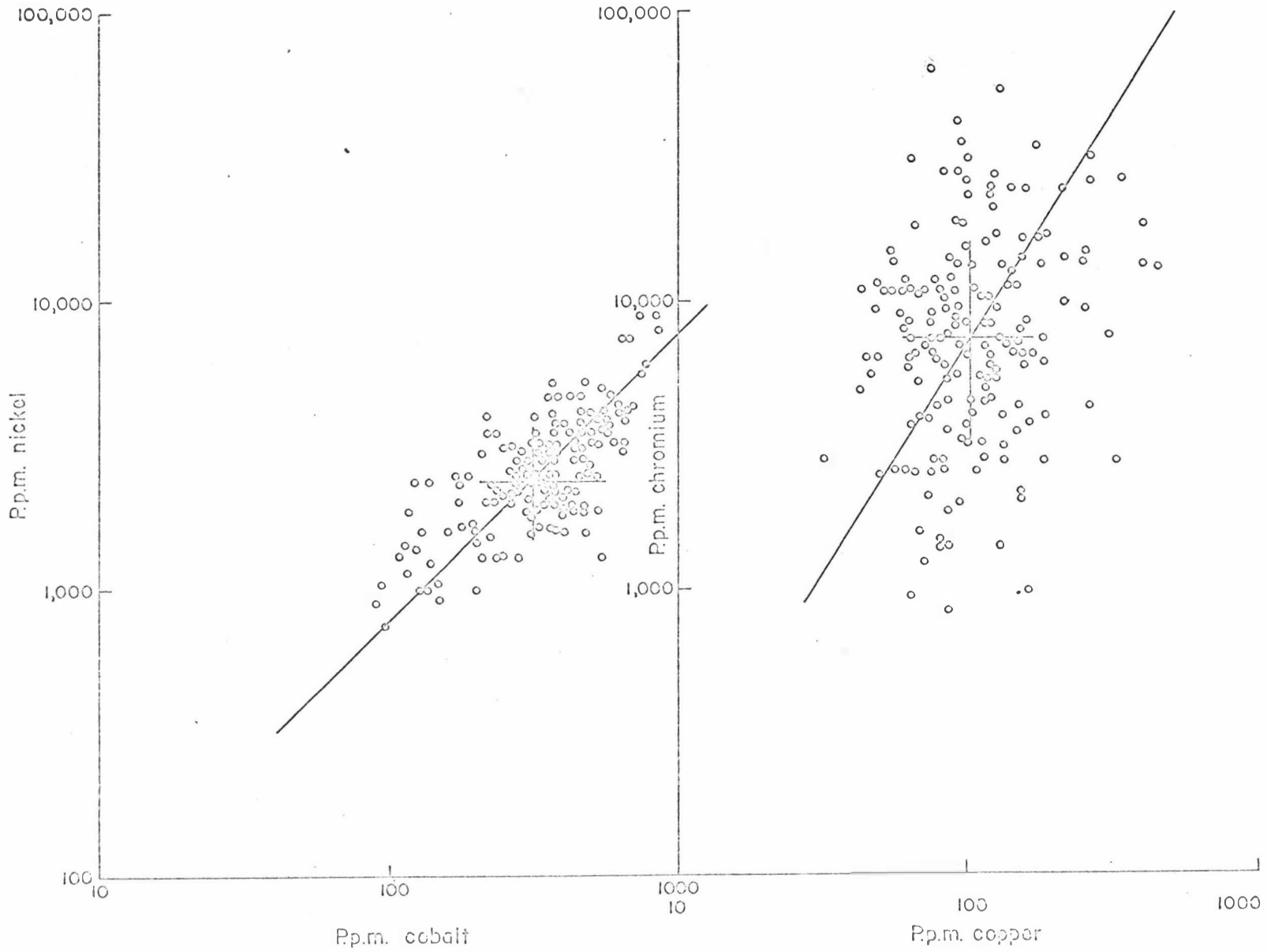

Fig. III - 4. Interelement relationships for soils only.

2.0 g of air-dried (minus-1 mm) soil was shaken for 16 hours with 20 ml of 2.5% acetic acid, pH 2.5, filtered and the solution analysed by atomic absorption spectrophotometry for chromium, nickel, copper, cobalt, calcium and magnesium under the conditions listed in Table III-1. Strontium nitrate solution was added before analysis for calcium to prevent interference by phosphate. The addition of strontium was found to have no effect on the analytical results for the other elements.

The results of these analyses, and the corresponding total trace element analytical results (corrected to ppm dry weight) are listed in Table III-15. Also listed are the means for these 24 samples. The total contents of calcium and magnesium were not measured.

The four trace elements showed different extractabilities. In terms of the total amounts of these elements present, the amounts extracted increased in the order: chromium ($< 0.1\%$), copper ($< 2\%$), cobalt (3.6%) and nickel (5.4%). In particular, only a very low proportion of the total chromium content was extractable. However, since plants do in fact accumulate greater amounts of chromium than nickel, it appears that this extractant is not an accurate indicator of the availability of chromium. Ammonium acetate solution (pH 7.0), ammonium chloride solution (pH 7.0) and distilled water were also used to extract one soil sample but in no case was there any detectable solubility of chromium.

As with chromium, copper was also undetected in the 2.5% acetic acid solutions, and only just measurable amounts of cobalt were found. However, quite large amounts of nickel were soluble in dilute acetic

Table III - 15

SOIL EXTRACT DATA

2.5% Acetic Acid Extraction for 16 hours
(ppm of dry soil)

Sample No.	Ash Content (%)	Cr Total	Cr Ex.	Ni Total	Ni Ex.	Cu Total	Cu Ex.	Co Total	Co Ex.	Ca Ex.	Mg Ex.	Ca/Mg
701	85.7	6200	<3	3500	147	158	<2	320	7	86	2250	0.038
702	80.4	1750	<3	2350	123	120	<2	370	10	105	2800	0.038
703	85.4	3600	<3	2800	161	175	<2	310	10	130	2250	0.058
704	81.3	3400	<3	2050	187	108	<2	290	12	105	2700	0.039
705	87.2	3800	<3	3100	161	130	<2	350	10	90	1900	0.047
706	83.7	3100	<3	2400	147	125	<2	285	10	78	2400	0.033
707	89.3	1900	<3	2050	127	90	<2	280	10	115	1900	0.061
708	89.4	12000	<3	1900	112	92	<2	240	10	78	2200	0.036
709	87.1	1850	<3	1750	113	81	<2	200	7	142	2300	0.062
710	86.1	2800	<3	2300	105	115	<2	240	10	86	2100	0.041
711	87.7	3300	<3	1900	95	87	<2	260	10	105	2000	0.053
712	84.5	5100	<3	2500	113	102	<2	300	12	98	2050	0.048
713	84.7	2400	<3	2500	80	112	<2	310	10	158	2500	0.063
714	90.6	2600	<3	3800	176	170	<2	450	10	133	1800	0.074
715	87.8	2500	<3	1900	131	70	<2	230	10	187	1600	0.117
716	70.3	2300	<3	1600	76	75	<2	180	7	221	3300	0.067
717	88.6	1350	<3	2300	151	72	<2	250	10	239	1650	0.145
718	84.6	2900	<3	2000	126	95	<2	260	12	220	2050	0.107
719	84.6	3900	<3	2100	135	90	<2	265	12	164	2250	0.073
720	87.0	3900	<3	2350	156	65	<2	200	10	126	2050	0.062
721	87.9	2400	<3	2800	156	84	<2	250	12	135	2300	0.059
722	82.6	4800	<3	2800	127	105	<2	380	12	143	2100	0.068
723	86.0	4000	<3	3000	100	92	<2	325	10	93	2050	0.045
724	90.6	6200	<3	2500	176	68	<2	250	12	105	1570	0.067
Mean	85.5	3670	<3	2430	132	103	<2	283	10.2	131	2170	0.062

acid, the average being the same as the average calcium extracted. The values recorded here may be higher than the exchangeable cobalt and nickel, for Mitchell (1945) showed that with change of pH from 2.5 (2.5% acetic acid) to 7.0 (normal ammonium acetate), the extractable cobalt and nickel decreased by a factor of ten, with little change in the extractable magnesium.

Recently, Ishihara et al (1968a, 1968d) have shown that the nickel contents of fruit trees on serpentine soils in Japan were highly correlated with the exchangeable nickel content of the soils. Ishihara et al (1968d) reported that chlorosis occurred in the leaves of Japanese pear trees (Pyrus sinensis) when the exchangeable soil nickel was greater than 2 ppm and the leaf content greater than 15 ppm. Both these values are exceeded by most plant and soil extract values in this thesis assuming a similar method of exchangeable nickel determination.

From the data, the extremely low availability of calcium compared to that of magnesium can be seen. The exchangeable calcium contents of these soils are similar to those in serpentine soils described by Robinson et al (1935). The magnesium values in Table III-15 are comparable with those described by Walker (1954) but Mitchell (1957) considered that 2.5% acetic acid extracted more magnesium than was truely exchangeable, especially from soils derived from basic igneous rocks.

(b) Plants

In this section, results are quoted and compared for all samples collected from serpentine soils. This includes 45 samples of C. vauvilliersii, 38 of H. odora, 47 of L. scoparium and 20 each of M. monroi, N. australe and P. suteri.

Table III - 16

ANALYSIS OF ALL SAMPLES

(concentration of ashed samples)

		Cr (ppm)		Ni (ppm)		Cu (ppm)		Co (ppm)		Ca (%)	Mg (%)	Ca/Mg
		Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Plant	Plant
C. vauvilliersii (39-45 samples)	Mean	525	6850	765	2070	97	87	37	300	8.83	4.28	2.06
	Std. Dev.	0.86	0.46	0.41	0.20	0.33	0.19	0.42	0.23	0.18	0.33	0.43
H. odora (31-38 samples)	Mean	166	6710	1240	2000	127	104	28	305	8.89	9.19	0.96
	Std. Dev.	0.66	0.42	0.31	0.19	0.23	0.17	0.29	0.25	0.14	0.31	0.43
L. scoparium (46-47 samples)	Mean	1180	7980	1270	2410	78	89	52	350	14.9	7.51	1.98
	Std. Dev.	0.65	0.34	0.45	0.21	0.26	0.18	0.45	0.19	0.13	0.19	0.28
M. monroi (20 samples)	Mean	680	6700	860	2570	45	100	42	330	3.77	2.88	1.31
	Std. Dev.	0.51	0.26	0.20	0.11	0.14	0.30	0.20	0.18	0.11	0.13	0.15
N. australe (20 samples)	Mean	510	12400	640	3900	18	105	83	390	5.21	14.2	0.36
	Std. Dev.	0.48	0.27	0.26	0.22	0.19	0.25	0.22	0.24	0.13	0.12	0.19
P. suteri (20 samples)	Mean	1800	12450	5860	3340	125	145	235	270	5.04	15.7	0.32
	Std. Dev.	0.54	0.32	0.23	0.18	0.20	0.23	0.26	0.23	0.16	0.10	0.22

The means and standard deviations for the samples are listed in Table III-16. These data for the elements chromium, nickel, cobalt and copper are also shown by the crosses on Figs. III-5 to III-10, which show plots of concentrations in plant ash against concentrations in soils.

The results show, as with the group samplings above, the much greater variation of concentrations in plant ash compared with the soil variation, as determined by standard deviation values. Generally, for chromium, the standard deviation for plant ash was nearly double that for the corresponding soils. The soils which supported the endemic species N. australe and P. suteri had a mean chromium content of 1.2% while all other soil means were less than 0.7% for chromium. This may be due to different criteria for collection of these species but compared with the random sampling, the only mean soil chromium content higher than 1.2% was that for H. odora. However, all species were collected from at least one soil sample with total chromium content greater than 2%.

The calcium and magnesium contents of plants differed considerably between species. L. scoparium, C. vauvilliersii and M. monroi were all (on the average) able to maintain an excess of calcium over magnesium despite the very much lower availability of calcium compared to magnesium (Table III-15). However, the absolute concentrations of these elements in these three species were quite different. M. monroi had half the concentration, and L. scoparium twice as much of both elements as C. vauvilliersii.

The other two endemic species, and the H. odora random samples, each had mean calcium contents of about 6% and magnesium about 15%, but overall, H. odora mean values were near to 9% for both calcium and magnesium.

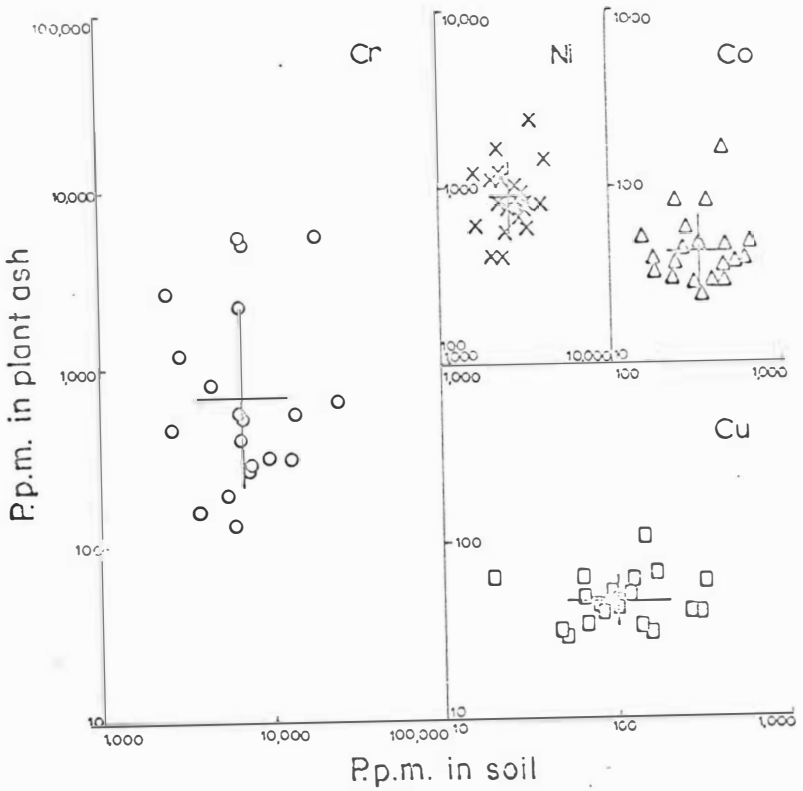

Fig. III - 5. Plant-soil relationships for Hycsotis monroi.

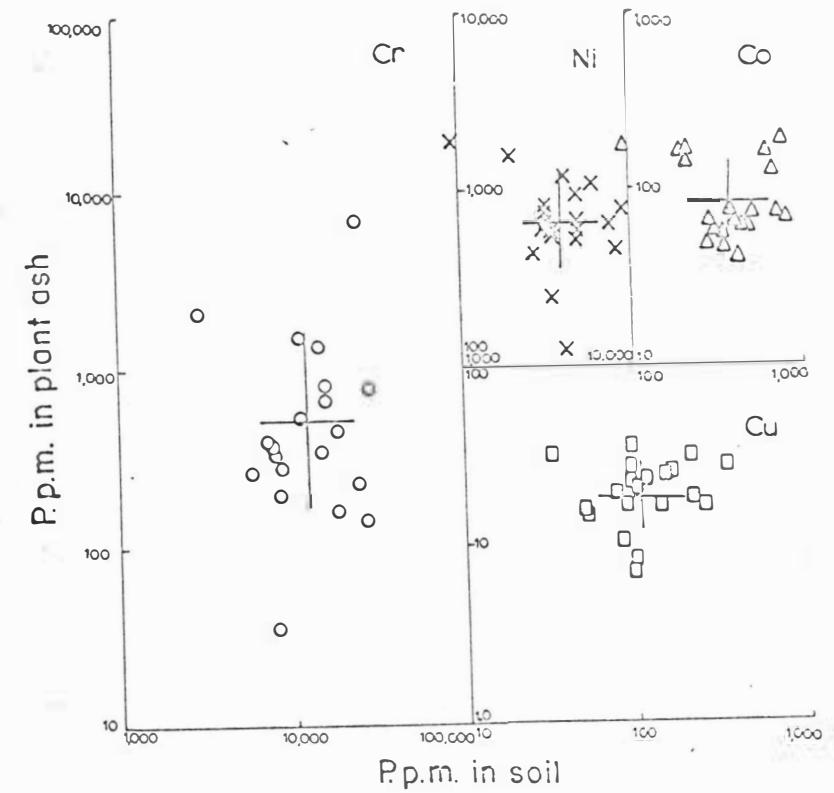

Fig. III - 6. Plant-soil relationships for Notothlasni australe.

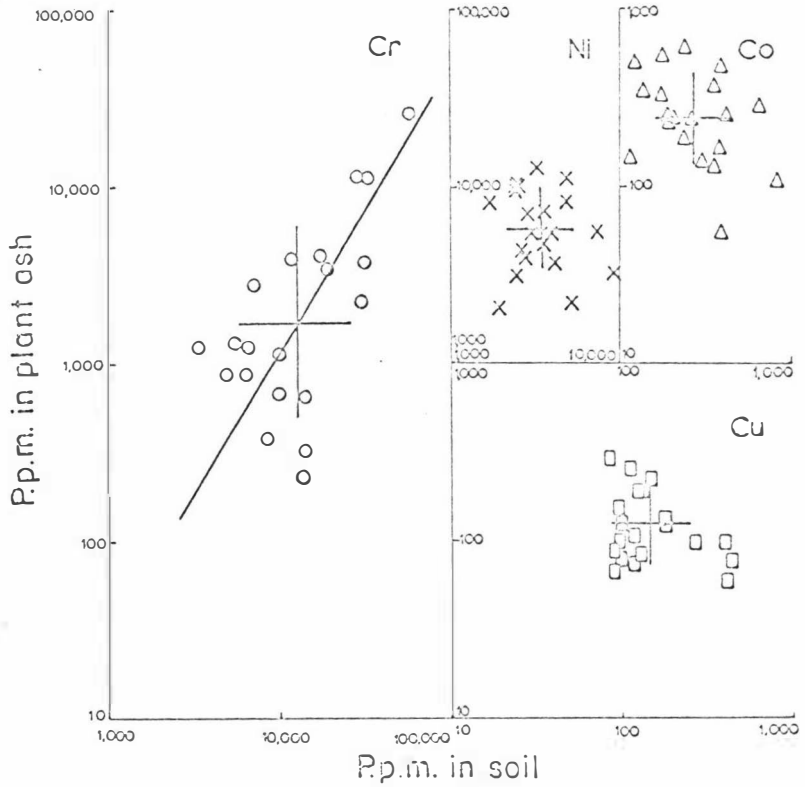

Fig. III - 7. Plant-soil relationships for Pimelea suteri.

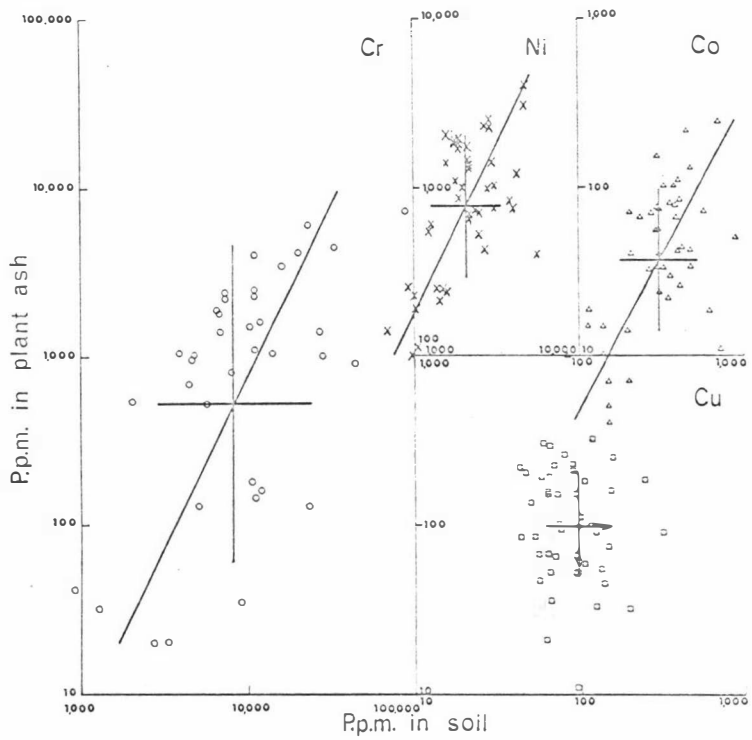

Fig. III - 8. Plant-soil relationships for Cassinia vauvilliersii.

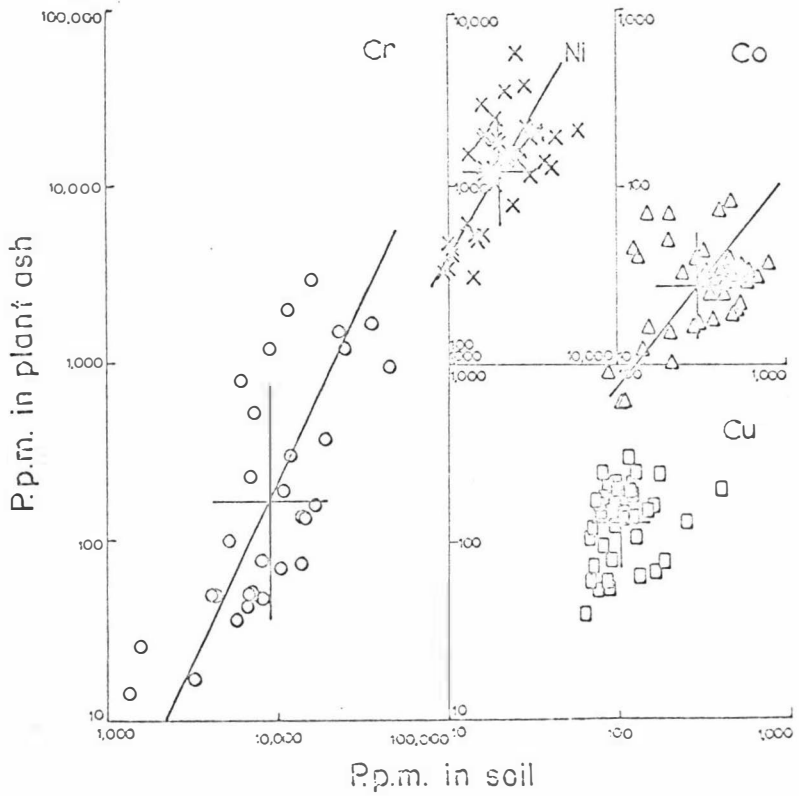

Fig. III - 9. Plant-soil relationships for Hebe odora.

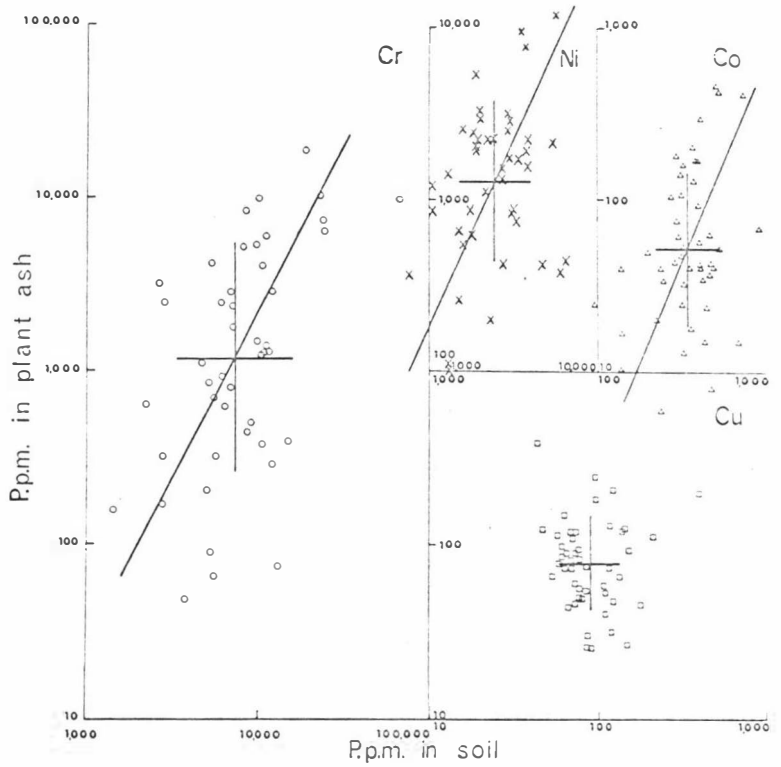

Fig. III - 10. Plant-soil relationships for Leptospermum scoparium.

Table III - 17

TRACE ELEMENT ACCUMULATION

Ratio of mean plant ash concentration to mean soil content

Species	Cr	Ni	Cu	Co
C. vauvilliersii	0.076	0.370	1.11	0.125
H. odora	0.025	0.620	1.22	0.092
L. scoparium	0.145	0.525	0.880	0.150
M. monroi	0.100	0.335	0.450	0.125
N. australe	0.041	0.165	0.170	0.215
P. suteri	0.145	1.75	0.855	0.870

Table III-17 shows the ratios of mean concentrations in plant ash to mean contents in soils for further comparison of the accumulative abilities of the six species for the elements chromium, nickel, copper and cobalt. For chromium, both L. scoparium and P. suteri had distinctive plant ash to soil ratios of 0.145, with the other species 0.10 or less. The ash of H. odora contained on the average only 2.5% of the chromium content of the soil. Even more significant was the accumulation of nickel and cobalt in P. suteri. The mean nickel concentration of this plant was almost twice, and the cobalt concentration almost equal to the corresponding levels in the soils. In each case, the ratio of ash content for P. suteri was more than three times that for the other species. For copper, only C. vauvilliersii and H. odora had a greater content in the ash than in their corresponding soils.

Table III - 18

PLANT - SOIL CORRELATIONS FOR ALL SAMPLES

	No. of samples	Cr	Ni	Cu	Co
C. vauvilliersii	39-45	+0.65 S**	+0.62 S**	-0.09 NS	+0.58 S**
H. odora	31-38	+0.72 S**	+0.63 S**	+0.27 NS	+0.35 S
L. scoparium	46-47	+0.45 S*	+0.31 S	-0.04 NS	+0.33 S
M. monroi	20	+0.05 NS	+0.20 NS	+0.10 NS	+0.08 NS
N. australe	20	+0.21 NS	-0.30 NS	+0.16 NS	-0.23 NS
P. suteri	20	+0.63 S*	-0.13 NS	-0.26 NS	-0.37 NS

Table III-18 shows the correlation coefficients for plant-soil relationships. The data are plotted in Figures III-5 to III-10 with the reduced major axes drawn on significantly correlated relationships.

Among the serpentine-endemic species, a highly-significant correlation ($r = +0.63$) between the plant ash and soil chromium contents for P. suteri was the only significant correlation. For the other three species, the results showed that for chromium, nickel and cobalt, often quite striking regularity of uptake occurred, suggesting that the concentration in the plant ash was dependent on the soil concentration. In particular C. vauvilliersii showed very highly significant ($P < 0.001$) correlations for each of the elements chromium, nickel and cobalt, while H. odora and L. scoparium showed

similar but less pronounced correlations. Chromium was correlated more strongly and for more species than the other elements studied. This result was probably enhanced by the wider variation of soil concentration for this element compared with the other elements, e.g. for chromium 800 - 90,000 ppm but copper 32 - 440 ppm.

The interelement relations between the concentrations of elements in the same plant were more complex. These data are only reported statistically (Table III-19). Among the endemic species, with only twenty samples of each plant, very few relationships were shown to be significant.

Significant correlations ($P < 0.05$) occurred between chromium and cobalt in all six species and to a lesser degree between nickel and copper in all six species. Very highly significant ($P < 0.001$) correlations were found for L. scoparium, C. vauvilliersii and M. monroi between nickel and cobalt, elements which were also very highly correlated in soils (Table III-14). Almost all the elements were correlated in pairs in H. odora to varying degrees.

Generally, in most plants, chromium, nickel, cobalt and magnesium concentrations increased together as calcium contents decreased (negative correlations). Less often, copper contents also increased with the other trace element contents.

Cumulative frequency diagrams for plant trace elements are shown in Figures III-11 to III-13. For chromium, both C. vauvilliersii and H. odora show significant changes in the slope of the graphs, indicating differences of the accumulation of this element above concentrations of 900 ppm and 1300 ppm respectively. For both H. odora and L. scoparium, the graphs for nickel also have changes of slope, and likewise for copper, those for C. vauvilliersii and H. odora indicate bimodal distributions. Other apparent variations

Table III - 19

INTERELEMENT CORRELATIONS FOR ALL SAMPLES

	C. vauvilliersii	H. odora	L. scoparium	M. monroi	N. australe	P. suteri
No. of samples	39-45	31-38	46-47	20	19-20	19-20
Cr - Ni	+0.74 S**	+0.34 NS	+0.75 S**	+0.35 NS	+0.42 NS	+0.66 S*
Cr - Cu	+0.16 NS	+0.39 S	+0.33 S	+0.15 NS	+0.55 S*	+0.58 S*
Cr - Co	+0.75 S**	+0.59 S**	+0.78 S**	+0.59 S*	+0.46 S	+0.51 S
Cr - Ca	-0.23 NS	-0.51 S*	-0.16 NS	+0.12 NS	-0.20 NS	+0.22 NS
Cr - Mg	+0.64 S**	+0.52 S*	+0.43 S*	+0.33 NS	-0.04 NS	-0.16 NS
Ni - Cu	+0.41 S*	+0.69 S**	+0.53 S**	+0.47 S	+0.51 S	+0.53 S
Ni - Co	+0.84 S**	+0.38 S	+0.88 S**	+0.75 S**	+0.20 NS	+0.45 S
Ni - Ca	-0.37 S	-0.66 S**	-0.22 NS	-0.08 NS	-0.69 S**	+0.44 S
Ni - Mg	+0.82 S**	+0.79 S**	+0.45 S*	+0.18 NS	+0.26 NS	-0.17 NS
Cu - Co	+0.39 S*	+0.43 S*	+0.55 S**	+0.39 NS	+0.27 NS	+0.17 NS
Cu - Ca	-0.20 NS	-0.70 S**	+0.10 NS	+0.27 NS	-0.28 NS	+0.23 NS
Cu - Mg	+0.32 S	+0.66 S**	+0.17 NS	+0.03 NS	-0.25 NS	+0.32 NS
Co - Ca	-0.37 S	-0.48 S*	-0.26 NS	+0.02 NS	-0.01 NS	-0.04 NS
Co - Mg	+0.76 S**	+0.51 S**	+0.33 S	+0.09 NS	-0.01 NS	-0.16 NS
Ca - Mg	-0.39 S*	-0.73 S**	-0.46 S**	+0.12 NS	-0.12 NS	-0.35 NS

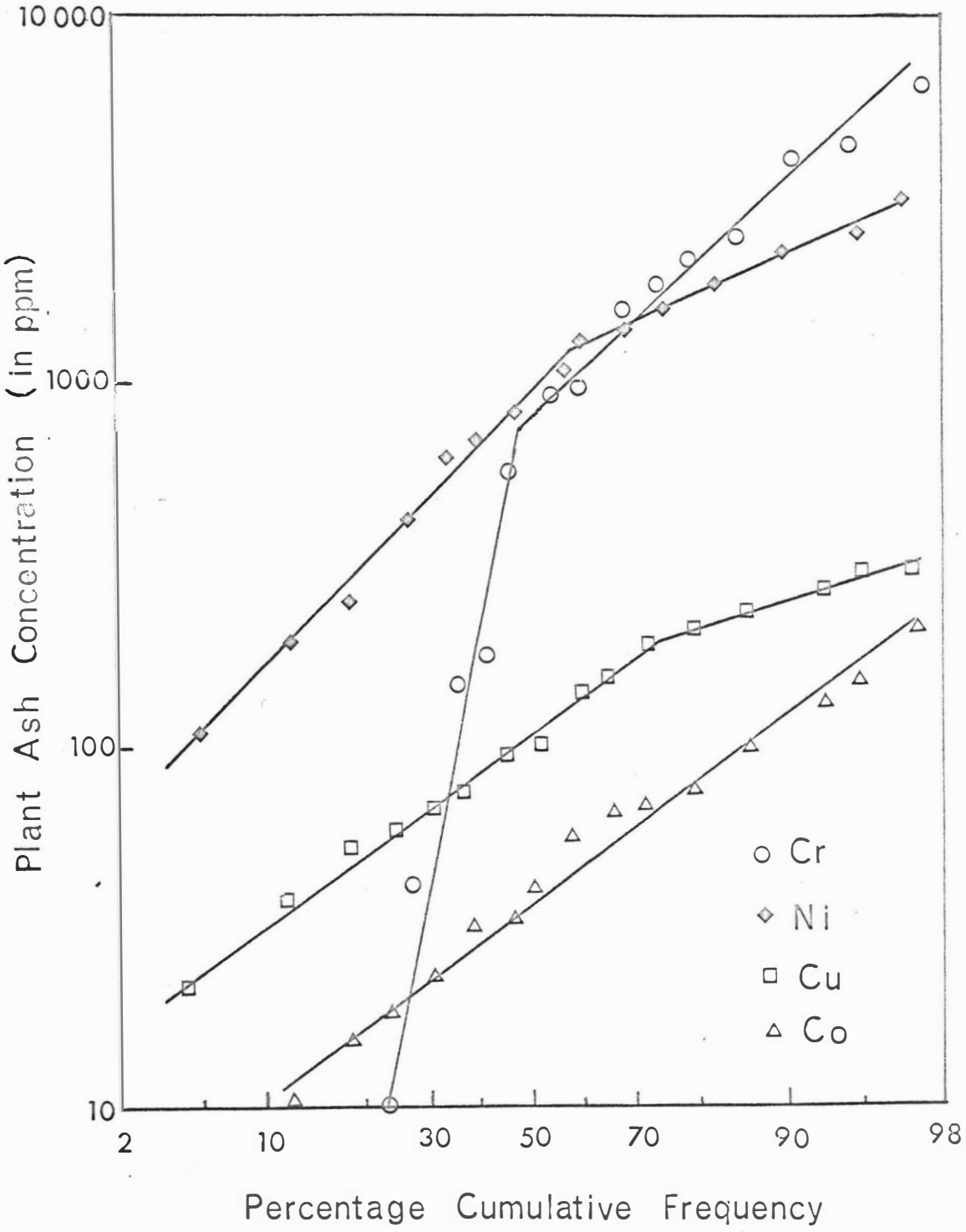

Fig. III - 11. Cumulative frequency diagram for Cassinia vauvilliersii.

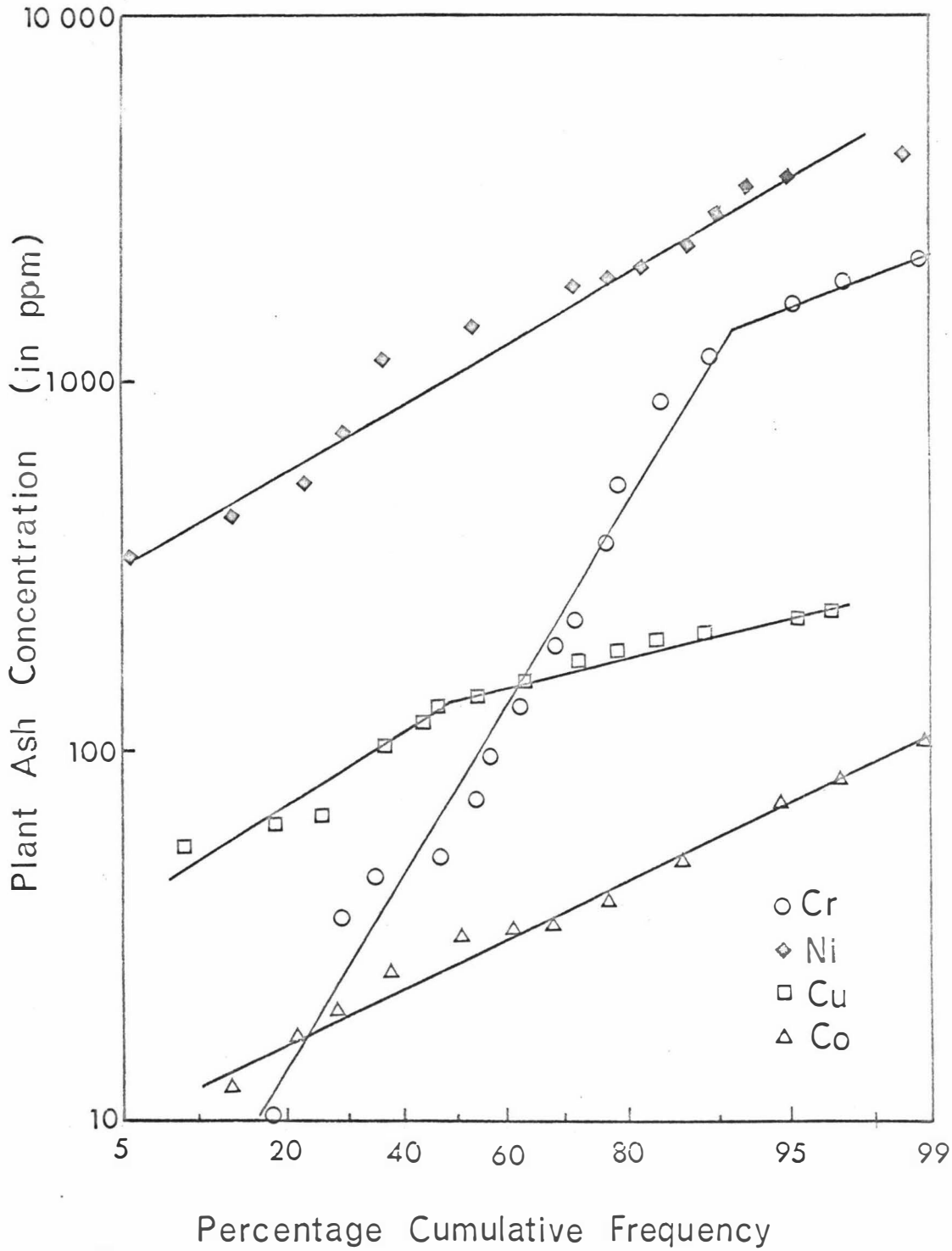

Fig. III - 12. Cumulative frequency diagram for Hebe odora.

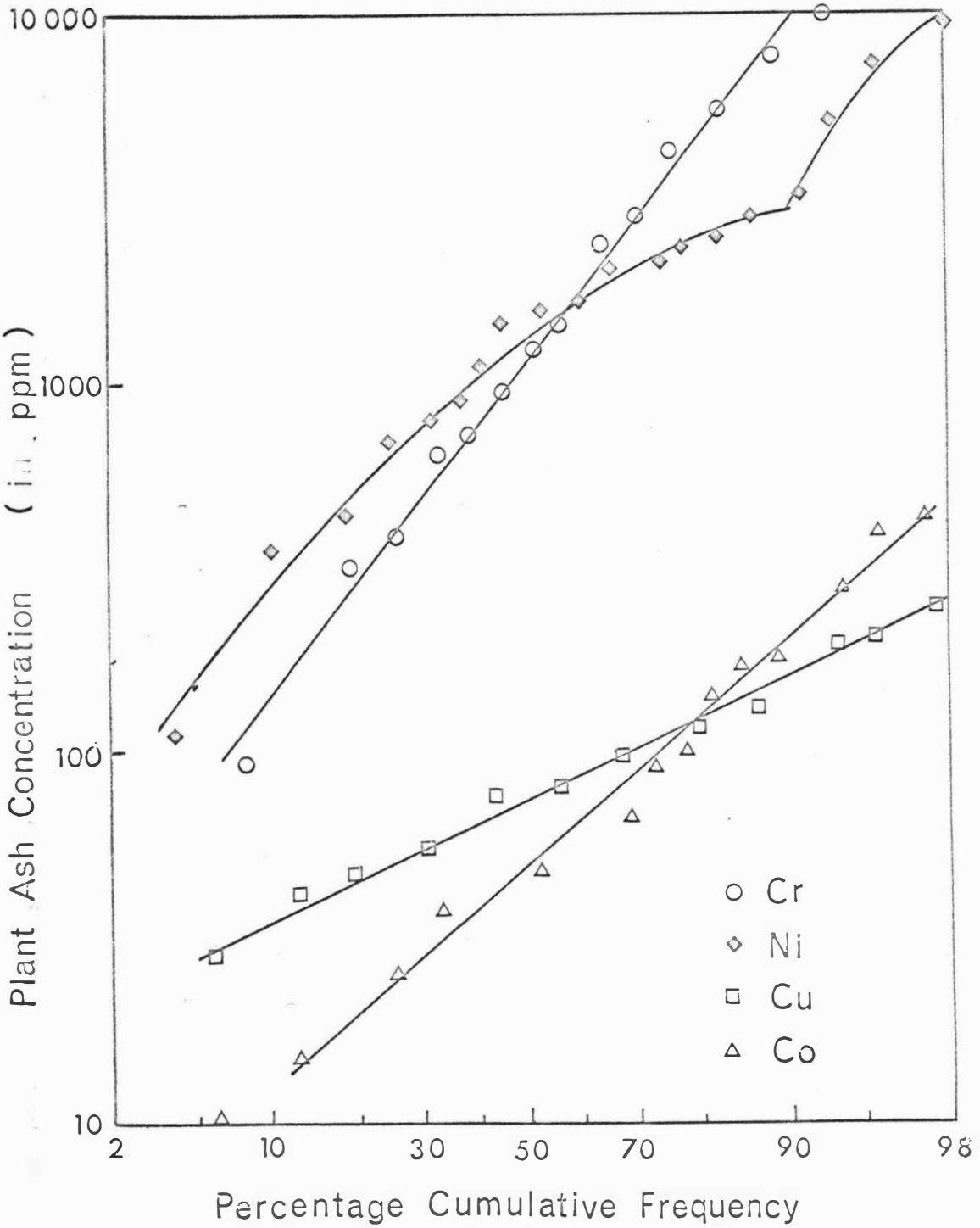

Fig. III - 13. Cumulative frequency diagram for Leptospermum scoparium.

from linearity are considered not significant because of the small numbers of samples considered here, less than 50 for each species. For the same reason, cumulative frequency plots for the other three plant species were not made.

6. GENERAL DISCUSSION AND CONCLUSIONS

Observations comparing flower colour of L. scoparium or pollen grain size for L. scoparium and C. vauvilliersii showed no apparent correlations with mineralisation in the Nelson Mineral Belt. Shacklette (1964) had reported changes in the flower colour of Epilobium angustifolium in the presence of radioactive ore in Alaska, and Malyuga (1964, p. 10) had observed changes of petal colour of Papaver commutatum due to mineralisation, and both plants were subsequently used for geobotanical prospecting. However, such changes as these appear to be rare in plants, and, in the limited study reported here, flower colour or pollen grain size appear to be unrelated to soil factors.

The tolerance of different species to unusual soils in New Zealand has also been shown by Wells and Whitton (1966), who reported that near hydrothermal activity, L. scoparium (manuka) is able to survive much closer to boiling mud pools than are pasture plants. L. scoparium is common throughout New Zealand in several types of communities (Cockayne, 1928) and has been able to adapt to unusual soil environments. In addition to its occurrence on the Nelson Mineral Belt, it forms large areas of scrub on weathered serpentine moraines in South Westland. Also, ferns are apparently adaptable to hydrothermal areas (Wells and Whitton, 1966), though rare on the Mineral Belt. The fern Asplenium viride is a chromium accumulator on Scandinavian serpentine areas (Lounamaa, 1956; Rune, 1953).

Biogeochemical prospecting methods were more promising than geobotanical methods in this serpentine area. C. vauvilliersii in particular could be used for either chromium, nickel or cobalt. In this species, for each of the three elements, there was a very highly significant relationship between the elemental concentration in plant ash and in the soil. H. odora would also be very suitable for biogeochemical prospecting for chromium and nickel, although for the same soil content, the chromium content of this plant was lower than in other species. Chromium could also be prospected using either P. suteri or L. scoparium in areas where these plant species grow.

It must be noted however, that these high plant-soil correlations in general hold over a wide range of concentration, and a large number of samples. If a smaller number of samples, or a narrower range of soil concentrations is taken, correlations may be less significant. Soil contamination, and hence misleadingly high correlations between concentrations in plant ash and soil, is unlikely to be important since plant samples were washed before analysis. The large calcium : magnesium ratios found, would be unlikely if serpentine soil contamination was significant.

Malyuga (1964, p. 78) described biogeochemical prospecting studies in the search for chromium, nickel and cobalt in the Southern Urals. He considered that, depending on the conditions of weathering, a plant survey will often be more useful than a soil survey for these elements in ultramafic environments. Thus, this section, as with earlier work (Part II of this thesis) shows that the biogeochemical method could be extended to New Zealand. It is notable also that, although the mean nickel content of soils is

about 2000 ppm, a value fifty times the average for soils (Hawkes and Webb, 1962), some species of plants were able to show significant plant-soil correlations and should be able to indicate anomalies or ore-bodies within the serpentine environment.

The work of Nicolls et al (1964-5) in Queensland showed that several species appeared to operate exclusion mechanisms for the elements copper and lead, although the accumulated zinc was proportional to the soil content. No evidence of exclusion mechanisms was found in this study.

A possible influence on the elemental content of these species, is a genotypic variation between samples from sites of different mineral content. Butler et al (1962) have shown that there can be significant heritability of the mineral contents of ryegrass. Also, Kruckeberg (1964) and Walker (1954) have shown that edaphic ecotypes exist within species which can occur on both serpentine and normal soils. Thus among the samples studied in the Nelson Mineral Belt, there could be a range of genetically-different strains of the same species, each adapted to its own level of soil metal content or with a specific elemental uptake. Where high correlation between the soil and plant concentrations of an element was found, however, it would appear that genotypic variation is a minor factor.

Various authors have reported many analyses of plants and soils from serpentine and other natural communities, but generally only representative soil samples have been considered (e.g. Paribok and Alexeyeva-Popova, 1966; Lounamaa, 1956; Gerloff et al, 1966). The present study, however, considered each plant in relation to its soil composition, and showed, for example, that for chromium in the Mineral

Belt, concentrations both in soil and plant ash can vary by three of four orders of magnitude. A comparison of elemental contents of different plant species must therefore take into account the corresponding soil concentration.

The concentration of chromium in the plant species studied here, ranged from less than 10 ppm to nearly 2% of the plant ash. In some specimens of H. odora, concentrations of chromium up to 2000 ppm in the ash were found, but only by comparing this with other plant species at the same soil elemental concentration can it be seen that H. odora consistently had the lowest chromium content of the six species studied. This difference, especially in relation to the high chromium contents of P. suteri and L. scoparium, can be seen more clearly by comparing the ratios of mean concentration in plant ash to mean content in soil. P. suteri and N. australe should be considered separately from the others as their soil samples contained an average of 1.24% chromium, twice the values for the other species, but the P. suteri mean chromium content was three times that of N. australe. These differences in chromium content indicate selectivity of uptake of chromium by these species.

Gerloff et al (1966), when studying native plants in Wisconsin, detected several instances of selective uptake of various elements. Potassium in some accumulator species was nearly four times the average content, while manganese appeared to be selectively excluded by one species from a bog high in manganese content. Other examples of accumulator plants are reported by Bowen (1966).

The investigations of Paribok and Alexeyeva-Popova (1966) and many others have shown that unusually high concentrations of chromium, nickel and cobalt are found in plants growing on ultramafic soils

(e.g. Birrell and Wright, 1945; Sarosiek, 1964). Rarely however have such high chromium contents been recorded as the 2.6% found in ash of a sample of P. suteri. The value of 9% chromium found in a soil on the Mineral Belt is also exceptionally high. Nemec (1954, 1957, quoted by Paribok and Alexeyeva-Popova, 1966) found that the content of chromium, nickel and cobalt attained levels of 2-4% in the leaves of some trees and considered that the destruction of trees on serpentine soils was due to the toxic effects of these metals.

In the samples considered here, nickel attained concentrations greater than 1.0% in only a few samples of P. suteri and L. scoparium. However, Vergnano (1958) found up to 3.5% nickel in the ash of the serpentine endemic species Alyssum bertolonii in Italy. Normal levels of nickel were found to be about 25 ppm in the ash of samples from Mount Egmont, but Wells and Whitton (1966) reported 3.1 ppm in dry matter (about 60 ppm in ash) in manuka (L. scoparium) samples from hydrothermally altered alluvium.

Lounamaa (1956) studied the trace element content of a large number of plants from ultramafic and other soils and found that the nickel and cobalt contents of deciduous trees, conifers and dwarf shrubs were generally similar to the values reported in this thesis. Ferns however, concentrated the elements chromium, nickel and cobalt more than did the other types of plants.

Rune (1953) concluded his vegetation study on serpentine floras of Northern Sweden by stating that nickel and chromium, present in high amounts, are the dominant causes of the peculiarity of the serpentine flora. Further, he gives special emphasis to the effect of nickel, but with insufficient evidence. He relies mainly on

the fact that more work had been done on the toxic effects of nickel than of chromium, and applies generalisations about the chemistry and physiological importance which could equally well be applied to chromium.

The concentrations of nickel, copper and cobalt in the serpentine plants showed much smaller variations than the concentrations of chromium, both between different species, and within any one species. The most outstanding finding was the accumulative ability of P. suteri with respect to chromium, nickel and cobalt. These high contents may be indicative of an unusual requirement for these elements, a factor which may be the cause of the endemism of P. suteri to serpentine soils. Certainly the tolerance to high levels of the elements chromium, cobalt and nickel must be a factor in the successful survival of endemic species in the serpentine environment.

Recently however, several authors have considered that the high amounts of chromium, nickel and cobalt are not such important factors as the high magnesium content and low calcium availability in serpentine soils with a consequent imbalance of these two elements in plants. In particular, Walker (1954), Kruckeberg (1954) and Walker et al (1955) studied the growth of various species including serpentine endemics, in various soil types. Normal soils and calcium chloride-leached serpentine soils produced better growth, especially of non-serpentine plants, than did serpentine soils. However, these papers do not give any complete soil analyses and it appears that the Californian serpentine soils contain lower amounts of chromium, nickel and cobalt than do most other serpentine soils.

Serpentine soils, from the Mineral Belt, when extracted with acetic acid solution, were found to have exchangeable calcium levels 10% or less of the values for exchangeable magnesium concentrations. Sarosiek (1964) described the ecological specificity of two serpentine soils from Lower Silesia. Exchangeable calcium and magnesium values were respectively 142 and 1045 ppm, amounts similar to those reported in this thesis. The total chromium, nickel and cobalt concentrations of 2%, 5200 ppm and 3400 ppm respectively are generally higher than those for soils near Dun Mountain, especially the cobalt content. However, the concentrations of calcium and magnesium in plants from both New Zealand and Poland show an ability of the plants to selectively reduce or overcome this soil imbalance. All plant samples had calcium : magnesium ratios greater than the values in soil extracts.

The degree to which the New Zealand plants can selectively accumulate calcium in preference to magnesium varies considerably between species. Also the total requirement of these two elements appears to vary for different species. Three of the species studied (L. scoparium, C. vauvilliersii, and M. monroi) had mean ratios of calcium to magnesium between 1.3 and 2.1 but their mean concentrations of these elements varied. M. monroi had an average ash content (for all samples) of only 3.8% calcium, compared with C. vauvilliersii 8.8% and L. scoparium 14.9%. Apparently these three species, each able to selectively accumulate calcium in preference to magnesium, had different requirements for these elements. M. monroi and N. australe should be considered separately from the other species, since these lack woody tissue which would require considerable calcium.

However, the two serpentine-endemic species, P. suteri and N. australe showed marked tolerances to a 3:1 excess of magnesium over calcium.

Walker (1954; Walker et al, 1955) showed that serpentine-endemic species were able to obtain more calcium than were other species from soils deficient in calcium. This appears to be the case in New Zealand where plants growing at the edge of the Mineral Belt had greater calcium : magnesium ratios than the same species sampled at Mount Egmont. Presumably those at the boundary of the serpentine area were serpentine-adapted ecotypes capable of very efficient extraction of calcium, and therefore when growing in soils only partly serpentine near the boundary, were able to attain high calcium : magnesium ratios.

Walker (1954) also showed that endemic and other plants grew better on serpentine soils when lime had been added. However, Hunter and Vergnano (1952) showed that with serpentinite soils from Scotland, the addition of calcium reduced the availability of the toxic element nickel due to a rise of pH. Ishihara et al (1968b, 1968c) considered that disease of Japanese fruit trees on serpentine soils was due to excessive absorption of nickel. This disease was alleviated by the application of slaked lime to soil and by foliar spraying with ammonium molybdate which reduced damage by nickel as well as easing the deficiencies of calcium and molybdenum.

Both Hunter and Vergnano (1953) and Ishihara et al (1968b) found that application of chromium to soils or nutrient solutions had little effect on the uptake of that element, whereas nickel addition increased nickel uptake. However, apparently calcium is also able to lessen the toxic effect of chromium on plants (Koenig, 1910,

quoted by Rune, 1953). Thus the effect of calcium on serpentine soils is an indirect one decreasing the availability of toxic elements, as well as directly increasing the availability of calcium. Liming would thus appear to be the most useful way to increase the fertility of serpentine soils.

The relationships between the various elements in the different species are often dissimilar. Although H. odora in general contained a much lower amount of chromium than the other species, on the average it had a calcium : magnesium ratio of 0.95, with both calcium and magnesium contents about 9% of the ash. On the other hand, L. scoparium had the highest mean chromium content and the highest mean calcium content (14%) with a calcium : magnesium ratio of 2.0. It appears from a comparison of these two species that the high calcium content of L. scoparium may be able to alleviate toxicity associated with the high chromium content of this species, whereas H. odora, rather than absorb more calcium, decreased its absorption of chromium.

The endemic species however, behaved differently. P. suteri had a very high chromium content, and the highest mean concentrations of nickel (5860 ppm) and cobalt (234 ppm) but the lowest calcium : magnesium ratio (0.32). It thus appeared that this species may have unusual requirements for the elements chromium, nickel, cobalt and magnesium, or alternatively, very efficient tolerance mechanisms. The other two endemic species have quite different mineral compositions to that of P. suteri. Both M. monroi and N. australe had much lower contents of chromium, nickel, copper and cobalt than the respective concentrations in P. suteri, but N. australe had almost the same calcium and magnesium levels. Also the mean soil concentrations for N. australe samples were similar to those for P. suteri whereas

the average chromium content of the soils supporting M. monroi was only half that corresponding to P. suteri. Possibly therefore, the different soils of lower chromium content, and probably also magnesium content, may account in part for the lower magnesium concentrations found in M. monroi. However, selective uptake must be used to completely explain the very low mean magnesium content (2.9%) of M. monroi, although requirement for the alkaline earth metals appears to be less for this species as the calcium content (3.8%) is also lower than in any other species.

All the species studied showed, as a direct response to the limiting factors of the serpentine environment, changes in the elemental compositions of the plants. All samples from the Dun Mountain Mineral Belt contained large amounts of the elements (chromium, nickel, cobalt and magnesium) which are at unusually high concentrations in serpentine soils compared with non-serpentine soils. Similarly plants generally contained lower amounts of calcium, an element deficient in serpentine soils. However, in addition to the mineral composition of the soil, the soil water supply, pH, and other ecological factors may be involved in the serpentine environment (Sarosiek, 1964).

The graphs of cumulative frequency plotted against concentration, in several cases show a change of slope, which may be interpreted in more than one way. A possibility is that the element concerned (chromium, nickel or copper) can exist in the soil in more than one form. Where an element could exist in more than one form, e.g. as different complexes, then a different form may predominate at high total soil concentrations to that at lower levels. These two forms may be assimilated by plants to different degrees, and hence produce

changes in the pattern of distribution of the element in plants. However this would be expected to apply to all plants whereas only some graphs show two distributions, and also, total soil contents show only one distribution on a cumulative frequency plot.

A more likely explanation is that at concentrations of the element in the soil above a certain level, a different mechanism of uptake or accumulation by the plant takes place. Two different mechanisms for uptake of many minerals have been found in several species (Epstein, 1966). However, in most cases considered here, the slopes of the cumulative frequency plot decrease at higher concentrations showing that the accumulation mechanism for the element concerned tends to accept the element less readily at higher concentrations.

A serious disadvantage of much of the research into biogeochemical prospecting has been the lack of attention paid to mechanisms of uptake, and sites of accumulation of trace elements by plants. Similarly, little work has been done on the tolerance of indigenous plants to mineralisation, considered from a nutritional point of view.

Further work was therefore carried out using radioisotopes in pot trials and to study the plant chemistry of various species. This would combine the aims of plant nutrition and biogeochemical prospecting and lay a firmer basis for the understanding of elemental accumulation by plants.

This work is described in the next part of the thesis.

PART IV

THE UPTAKE AND METABOLISM OF CHROMIUM-51

1. INTRODUCTION

In Part III, it was shown that most of the plants from the serpentine area had accumulated far greater amounts of chromium than had plants which grew near the boundary of this region, or those from Mount Egmont. Although nickel and cobalt were also present in greater amounts in serpentine plants than in others, the concentrations of these elements were lower than the concentrations of chromium. Also, these other elements did not occur in such a wide range of concentrations as did chromium which was found to occur in amounts between < 10 ppm and 27,000 ppm in ash of some plants. It was also shown in Part III that the plants studied were more useful for biogeochemical prospecting for chromium than for any of the other elements. Chromium, usually at low concentrations in plants, thus assumes a greater importance in plants from serpentine soils.

The chemistry of chromium in plants has been studied very little, although chromium occurs widely in the botanical world (Grosman, 1966; Pratt, 1966; Saint Rat, 1948). The earliest work with chromium was aimed at increasing crop yields by the addition of chromium salts to soil. Voelcker (1923), with pot trials found that 0.005% chromium as potassium chromate added to soil was toxic to plants during the first season after application, but that in the second season, a stimulating effect was observed. Similar studies in the field have continued, especially in France, where Bertrand and de Wolf (1968) obtained an optimum 42% increase in the yield of potatoes, when an addition of 40 g/ha of soluble chromium (as chrome alum) was made. But these claims may not be justified since few experimental details were given, and no statistical data were presented in these papers.

Sand and water culture experiments such as those of DeKock (1956) and of Hunter and Vergnano (1953) have shown that toxicity symptoms of chlorosis and necrosis occur in mustard plants at 2 ppm chromium (as $CrCl_3$ or Cr-EDTA complex) in solution culture, and in oats and barley at 10 ppm chromium (as potassium dichromate) in sand culture, all with increased leaf phosphorus levels. The unhealthy leaves had chromium contents of 15-65 ppm in the ash compared with 2 ppm in the ash of control plants, but the root ash contained 20,000 ppm chromium.

Hewitt (1963) showed that the effect of molybdenum on the chromium-induced chlorosis depended on whether chromium was fed as chromate or as chromium (III). Scharrer and Schropp (1935) and Gericke (1943) have both reported that chromate is more toxic by a factor of ten, than is chromium (III) when fed to plants. In none of these studies was the actual form of chromium looked at in plants, nor has chromium been shown to be essential to plants (Hewitt, 1963).

Recently however, Schroeder (1968) has considered chromium to be essential for glucose metabolism in rats, and it is also involved in glucose tolerance in humans (Anon, 1968). Its importance at low amounts for animal and human nutrition, therefore increases the need for information on chromium as it occurs in plants.

As very little chemical work has been done on chromium in plants, it was decided to carry out isotope studies using the isotope ^{51}Cr, which is readily available and of convenient half-life and activity. A radioisotope study is the most feasible method of attempting to find out in what forms of combination a trace element occurs in a plant, its mechanism of absorption and its movement within the plant. It may even be possible to determine why the element is present, and

how it is able to be accumulated in amounts which may be determined by the soil concentration.

The only previous isotope study of chromium in plants is the recent work of Bourque et al (1967) who studied the uptake of ^{51}Cr as an indicator of metabolic change in wheat root tips. They concluded that a greater uptake of ^{51}Cr in plant cells occurred when an increase in the rate of cell metabolism had been induced by vernalisation. Their study, however, considered only root sections, unlike the present work where the uptake was by intact plants. Bourque et al (1967) also concluded that only hexavalent chromate was able to penetrate intact cells, and not trivalent chromium. However, their method of feeding ^{51}Cr in the trivalent state, was to treat the root tissue with an excess of sodium ascorbate before incubation with ^{51}Cr-sodium chromate in order to reduce the chromate to the chromic state. It is possible that the presence of ascorbate had an effect on the uptake of ^{51}Cr.

The present studies were commenced in 1966, before the work of Bourque et al (1967) had been published, and as there was no other literature available on the chemical form of chromium taken up by plants, some of the studies were carried out in duplicate with both sodium ^{51}Cr-chromate and ^{51}Cr-chromic chloride solutions.

2. MATERIALS AND METHODS

(i) Chemicals

All chemicals for nutrient solutions and chemical separations were analytical grade or redistilled where possible, except for some of those used only in trace amounts.

(ii) Radiochemicals

Chromium-51 was obtained from the Radiochemical Centre, Amersham, England as either sodium ^{51}Cr-chromate or ^{51}Cr-chromic chloride. Sodium ^{51}Cr-chromate was supplied as an isotonic solution without a bactericide, whereas ^{51}Cr-chromic chloride was as a sterile isotonic solution. The volume and specific activity varied from batch to batch, but most batches contained about 1 mCi per ml and 5 to 10 µg chromium per mCi, although 1968 supplies contained 10 mCi per ml.

(iii) Measurement of Chromium-51

Chromium-51 has a half-life of 27.8 days and decays by electron capture, emitting gamma-rays of 0.323 MeV energy. This radiation was detected using Philips counting equipment with a 1½ inch diameter thallium-activated sodium iodide crystal in a Philips PW4111 scintillation head. Either of four systems was used:

(a) lead castle with manual sample change for planchets

(b) automatic sample changer PW4001, for up to 40 planchets, with time printout after presetting counts.

(c) tripod support for scintillation head, allowing tubes of radioactive sample to be placed over the detector.

(d) strips of paper from a chromatogram or electrophoretogram could be scanned by passing them under the scintillation head fitted with a lead collimator. The rate meter readings were recorded on a chart whose speed was synchronised with the paper strip.

(iv) Radioautography

Radioautographs were made by contacting the active tissue or paper, with Kodak Medical X-ray Film, obtained as sheets 43 cm x 35 cm. Radioautographs of electrophoretograms and chromatograms were scanned using a double-beam recording microdensitometer (Joyce, Loebl and Co. Pty Ltd). This method produced scan patterns with much better resolution than those from direct scanning with the scintillation detector.

(v) High Voltage Electrophoresis

Separation of the components of plant extracts was carried out using a high voltage electrophoresis apparatus (Miles Hivolt Ltd). Whatman 3MM paper, cut to 43 cm x 53.5 cm, was dipped in buffer, and uniformly blotted before application of up to eight samples to the centre of the sheet. These samples were applied as 1.25 cm bands by a glass micropipette. The experimental conditions were

(a) pH 5.3 in pyridine/acetic acid buffer (Efron, 1960), 10 minutes at 300 mA and 5.8 kV.

(b) pH 2.0 in formic acid/acetic acid buffer (Efron, 1960), 10 minutes at 300 mA and 4.8 kV.

(vi) Plant Culture

Plants were grown in a growth cabinet which was kept at a day temperature of 18°C (65°F) with a light intensity of 2000 ft-candles. There was a 6 hour dark period daily at a temperature of 13°C (55°F).

The nutrient solution had the following composition: 2.0 mM NH_4NO_3, 3.0 mM $(NH_4)_2SO_4$, 2.0 mM $Ca(NO_3)_2$, 2.0 mM $MgSO_4$, 2.0 mM KNO_3, 1.0 mM K_2HPO_4, 3.0 ppm iron as Fe-EDTA, 0.5 ppm boron as H_3BO_3, 0.5 ppm manganese as $MnCl_3$, 0.05 ppm zinc as $ZnSO_4$, 0.05 ppm molybdenum as Na_2MoO_4, and 0.02 ppm copper as $CuSO_4$. The pH of this solution was 7.3.

After feeding plants with radioisotope, roots were severed from shoots (and in the case of woody plants, branches from stems), placed in aluminium foil trays and were freeze-dried in a drum freeze-drier. For radioautography, the plants were humidified before glueing to drawing paper with PVA adhesive, which was allowed to dry while the plants were in a botanical press in contact with a smooth surface.

3. EXPERIMENTS WITH RED CLOVER, TRIFOLIUM PRATENSE

For the initial uptake studies with ^{51}Cr, red clover was used as it was readily available as seed, and was quickly and easily grown in nutrient culture for ease of working. The plants from serpentine areas were relatively slow growing and were not available as seeds. A common plant such as clover also serves as a reference plant for comparison with native species.

The seed used was of red clover, Trifolium pratense, L. (Leguminosae), 1966 Grasslands Turoa Government Stock D 1164 obtained from Grasslands Division, D.S.I.R., Palmerston North.

(i) Conditions of Culture

The clover seed was sown on glass beads dampened with distilled water, in an initially enclosed dish, and placed in the growth cabinet. After 14 days growth, five seedlings were transferred to each two-litre pot containing two-thirds strength nutrient solution. This nutrient solution was changed weekly. Feeding of ^{51}Cr and subsequent harvesting of the plants was carried out when the seedlings were between 25 and 40 days old.

Partial analyses of samples of clover are shown in Appendix 3.

(ii) Radioautographic Studies

An initial experiment was carried out with 24 clover seedlings, growing in nutrient solution four to a pot. To each pot was added 50 μCi of ^{51}Cr. Three pots received ^{51}Cr-chromic chloride and another three sodium ^{51}Cr-chromate, mixed with the nutrient solution. The plants remained in this solution for eight days before being harvested and freeze-dried.

Radioautographs of these plants showed that there was only radioactivity in the roots, and none in the aerial parts. No differences were apparent between those plants fed Cr^{3+} and those fed CrO_4^{2-}. Thus, it appeared that the ^{51}Cr might have been precipitated at the roots or in the solution instead of having been taken up by the plants, the precipitation being caused possibly by phosphate or other ions in the nutrient solution. In order to prevent any likelihood of this happening, future experiments were carried out by washing the nutrient from the roots for an hour or more with several changes of distilled water before placing the plants in a solution of only the radioisotope in distilled water. However, in all experiments, the roots always had a very much greater activity than the shoots.

An experiment was undertaken to determine if ^{51}Cr moved preferentially into young actively-growing leaves. Several clover plants, 35 days old, were washed in distilled water and then divided into groups, each with four plants to 30 mls of solution containing 200 μCi ^{51}Cr. After 24 hours they were washed again and returned to nutrient solution without added chromium. Plants were withdrawn at times of 1 day, 2 days, 4 days and 8 days after the commencement of the ^{51}Cr feeding. These were then radioautographed. Figure IV-1 shows a typical plant after 1 day, i.e. 24 hours in ^{51}Cr and not returned to the nutrient solution. Most of the leaves, including the youngest (arrowed) contained approximately the same amount of radioactivity. Figure IV-2 is of the shoots and roots of a clover plant which, at the same time as that in Figure IV-1, had had its youngest leaves tagged (here arrowed), but was harvested after 8 days, i.e. a further 7 days in nutrient solution elapsed before harvesting. Comparison of the radioautograph with the photograph

Fig. IV - 1. Red clover seedling after 24 hours in ^{51}Cr solution. Arrows indicate young leaves at the start of the experiment. Left : photograph. Right : radioautograph.

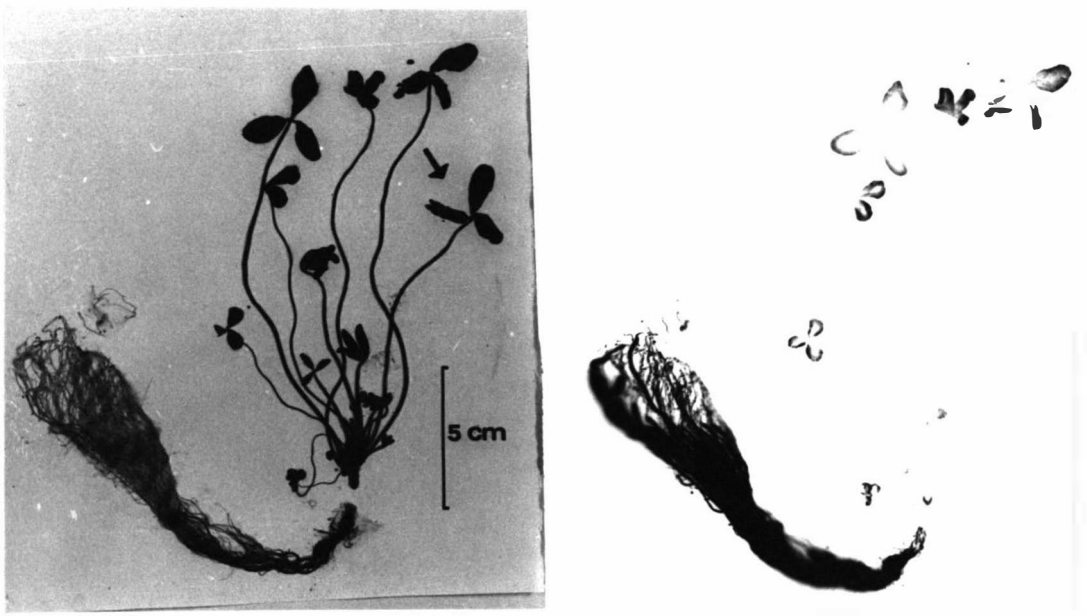

Fig. IV - 2. Red clover seedling after 24 hours in ^{51}Cr solution followed by 7 days in nutrient solution. Arrow indicates a young leaf at the start of the experiment. Left : photograph. Right : radioautograph.

of the plant shows that the arrowed leaf, much more mature than when marked, is of a similar or lower activity compared with the other mature leaves of the plant.

The youngest leaves in Figure IV-2 had very little activity in them. These leaves had formed and grown after the plant had been removed from the ^{51}Cr solution showing that although the roots still contained very much greater amounts of chromium, this was not available to new growth after transfer of the plants from the ^{51}Cr solution to nutrient solution. Thus, it seemed that most of the ^{51}Cr was immobilised in or on the roots initially. It may be that the presence of the nutrient solution, with phosphate, sulphate, iron-EDTA and other ions, is able to cause precipitation and immobilisation of ^{51}Cr at the roots.

The shoots of the plants which were supplied with $^{51}CrO_4^{2-}$ appeared to be of lower activity than those supplied with $^{51}Cr^{3+}$, although there appeared no significant differences between the corresponding root activities. However, both the experiments with $^{51}Cr^{3+}$ and with $^{51}CrO_4^{2-}$ showed similar distributions and the illustrations are of those fed $^{51}Cr^{3+}$.

These experiments show therefore that there is no advantage to be gained by allowing the plants to grow for a longer period in nutrient solution without added ^{51}Cr to attempt to get more ^{51}Cr into the shoots. Rather, this would merely dilute the ^{51}Cr by increasing the amount of non-active tissue.

Radioautographs of the upper portions of clover plants show, as did activity measurement, the very low levels of ^{51}Cr activity compared with the roots. The shoots usually had about 1 to 3% of the total radioactivity but 35-80% of the freeze-dried weight.

In general, the petioles as well as the leaves showed activity. Most of the leaf radioactivity was at the edges as shown in the enlargement (Figure IV-3) and was often at higher concentrations in the veins of the leaves than the inter-veinal areas, though occasionally this seems to be reversed as in the lower leaves of Figure IV-4. Older, dying leaves, such as the upper one in Figure IV-4 are most radioactive at the very edges, but have blotches distributed unevenly over most of the leaf.

To ensure that the freeze-drying of the plants did not play any part in the movement of ^{51}Cr to the edges of the leaves, a further experiment was carried out. These plants, which were fed similarly to the above, had the leaves separated from the petioles and from each other before freeze-drying, but radioautographs showed identical features to those described above.

(iii) Uptake Studies

An experiment was carried out, again comparing $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$, but also comparing the amount of ^{51}Cr translocated to the shoots, as a function of the activity in the roots, for different lengths of time of feeding the ^{51}Cr solution. There were four groups of five plants (27 days old) in the experiment, two groups were fed $^{51}Cr^{3+}$ and two fed $^{51}CrO_4^{2-}$. Each of the plants, which had been chosen for uniformity, was washed in distilled water for two hours and then each group was placed in a solution containing 750 μCi of ^{51}Cr in 30 mls of distilled water. One group of five plants was removed from each of the $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ solutions, after 2 hours and the other groups after 12 hours, each being then washed and freeze-

Fig. IV - 3. Enlarged radioautograph of red clover leaves showing ^{51}Cr radioactivity in the veins.

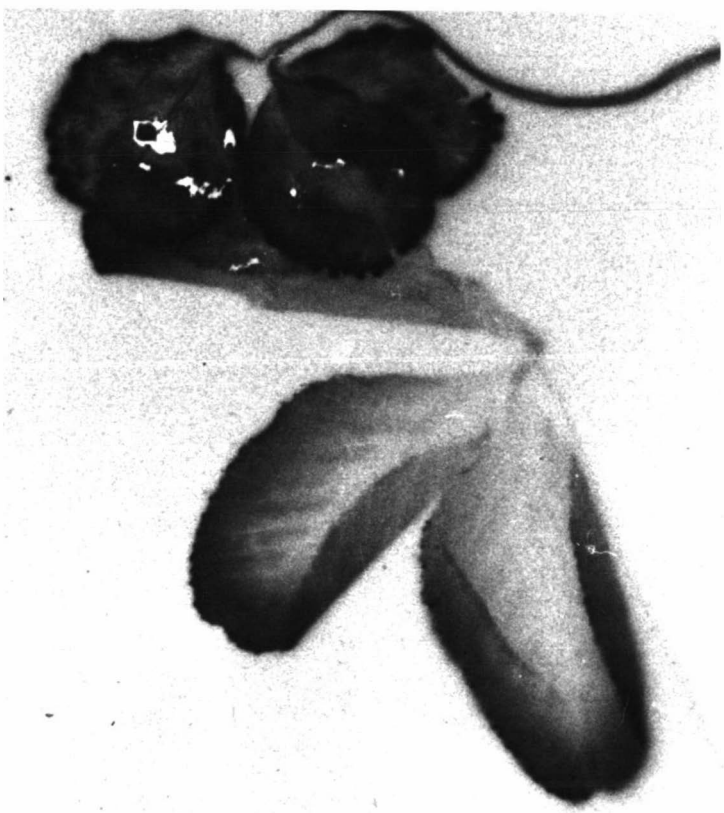

Fig. IV - 4. Enlarged radioautograph of red clover leaves showing ^{51}Cr radioactivity in interveinal regions of lower leaf and accumulations at margins and in islands in the upper (senescent) leaves.

dried. The dried plant material was weighed and then counted on planchets in the automatic sample changer.

Table IV-1(a) presents the results and Table IV-1(b) gives values of Students 't' computed from the shoot:root ratios by comparing groups of five plants with each other. These tables show that there were no differences (indicated by $t < 2.31$) between the shoot:root ratios of activity of the two sets of plants fed ^{51}Cr, either on a basis of total counts per part of the plant, or on specific activity. However, for the plants fed for 12 hours, a value of $t > 3.36$ shows that on a specific activity basis there was a difference, significant at the 1% level, between the two groups of plants fed different forms of chromium. Similarly, on a total count basis, there was a significant difference at the 5% level.

In all cases, comparison of the two hour feeding with the twelve hour feeding, shows that 12 hours allowed more activity to be incorporated in all parts of the plant. This increase varied from about 10% in the Cr^{3+}-fed roots to 200% in the Cr^{3+}-fed shoots, with the roots and shoots of the plants supplied with CrO_4^{2-} intermediate in value. There were significant increases in the shoot: root ratios in most cases. These showed that more of the ^{51}Cr was able to be translocated if allowed time. In all cases the radio-activity in the shoots was between 0.1 and 1.1 percent of that in the roots.

The plants fed Cr^{3+} for 12 hours had a shoot:root activity ratio, of either total counts or of specific activity, which was about twice that of the plants fed CrO_4^{2-} for the same time. This was mainly due to an increase in the activity of the roots of the chromate-fed plants, rather than the increased translocation to the shoots by the plants fed Cr^{3+}, which also occurred. However,

Table IV - 1

a) CHROMIUM-51 RADIOACTIVITY IN CLOVER

(Each value is the mean of five plants)

		2 hours			12 hours		
		Shoots	Roots	Shoot/Root	Shoots	Roots	Shoot/Root
Total Activity (c/m)	CrO_4^{2-}	3447	866.3×10^3	4.02×10^{-3}	7538	1226.8×10^3	6.13×10^{-3}
	Cr^{3+}	2901	742.3×10^3	4.10×10^{-3}	9055	860.8×10^3	10.83×10^{-3}
Specific Activity (c/m/mg)	CrO_4^{2-}	50.66	33.09×10^3	1.50×10^{-3}	86.54	52.65×10^3	1.69×10^{-3}
	Cr^{3+}	35.78	23.30×10^3	1.55×10^{-3}	95.10	26.28×10^3	3.79×10^{-3}

b) VALUES OF STUDENT'S "t" FOR COMPARISONS

(For 8 degrees of freedom, critical values of "t" are 5% 2.31, 1% 3.36)

Comparing:	2 hr. Cr^{3+}	12 hr. Cr^{3+}	CrO_4^{2-} 2 hr.	Cr^{3+} 2 hr.
with:	2 hr. CrO_4^{2-}	12 hr. CrO_4^{2-}	CrO_4^{2-} 12 hr.	Cr^{3+} 12 hr.
Total Activity	0.14	3.29	2.97	4.91
Specific Activity	1.08	3.50	0.76	3.83

these results agree with the observations of radioautographs which showed less activity in the chromate-fed shoots.

The above comparisons depend on uniformity of the plants between the groups compared. No significant differences between the weights of these groups of plants were detected by 't' test.

The differences shown here between the plants which were fed with $^{51}Cr^{3+}$ and those fed $^{51}CrO_4^{2-}$ are not in agreement with the observations of Bourque et al (1967). Their cut wheat roots showed a decrease of 93% in the uptake of ^{51}Cr fed as Cr^{3+} (produced by treating the CrO_4^{2-} with ascorbate) compared with CrO_4^{2-}. The remaining 7% they attributed to absorption of Cr^{3+} on the cut surfaces, so that the actual uptake of Cr^{3+} is negligible. This is shown here not to be true in the case of clovers which are able to absorb and translocate to the leaves substantial amounts of both Cr^{3+} and CrO_4^{2-}. More especially, the Cr^{3+} form is apparently able to be translocated to the shoots more readily or faster than is CrO_4^{2-} over a 12 hour period, but $^{51}Cr^{3+}$ is less able to be absorbed by the roots than is $^{51}CrO_4^{2-}$.

(iv) Chemical Fractionation of Clovers

Bourque et al (1967) considered that a large proportion of the ^{51}Cr which was taken up by wheat seedling roots in their experiments, was bound to the proteins, but there appear to be no other references in the literature to the site or chemical state of chromium in plants. However, other trace elements such as zinc (Diez-Altares and Bornemisza, 1967), selenium (Peterson and Butler, 1962, and others) and iron and manganese (Tiffen, 1967, and others) have been studied in more detail, and Bowen et al (1962) studied a wide range of elements in tomatoes.

To study further the state of binding of chromium in clover, a successive extraction system based on that of Bowen et al. (1962) was used, as indicated in Figure IV-5. Fractions A and A_1 were separated only for shoot material. Each liquid fraction was concentrated under vacuum in a rotary film evaporator at $<40^{\circ}C$, or was freeze-dried. These concentrates, and the residue, were transferred to aluminium or glass planchets for activity determination. The automatic sample changer system was used to record the time for at least 1000 counts to occur.

Some of the substances which occur in the fractions listed in Figure IV-5, are:

A	amino acids, lipids
A_1	organic acids, pigments
B	ionic and polar compounds
C	ionic and polar compounds
C_1	pectates, proteins
D	polar compounds
D_1	nucleic acids
E	degraded proteins, polysaccharides
F	cellulose, lignin

Twenty clover plants (35 days old) which had been selected for uniformity, were divided into two groups, and were removed from the nutrient solution. After the roots had been washed, the plants were transferred into distilled water which contained 350 μCi ^{51}Cr as the appropriate chemical form in 30 ml water, so that 10 plants received sodium ^{51}Cr-chromate and 10 received ^{51}Cr-chromic chloride. Further water was added as required, and after 24 hours metabolism in the ^{51}Cr solution, the roots were again washed and then returned to the

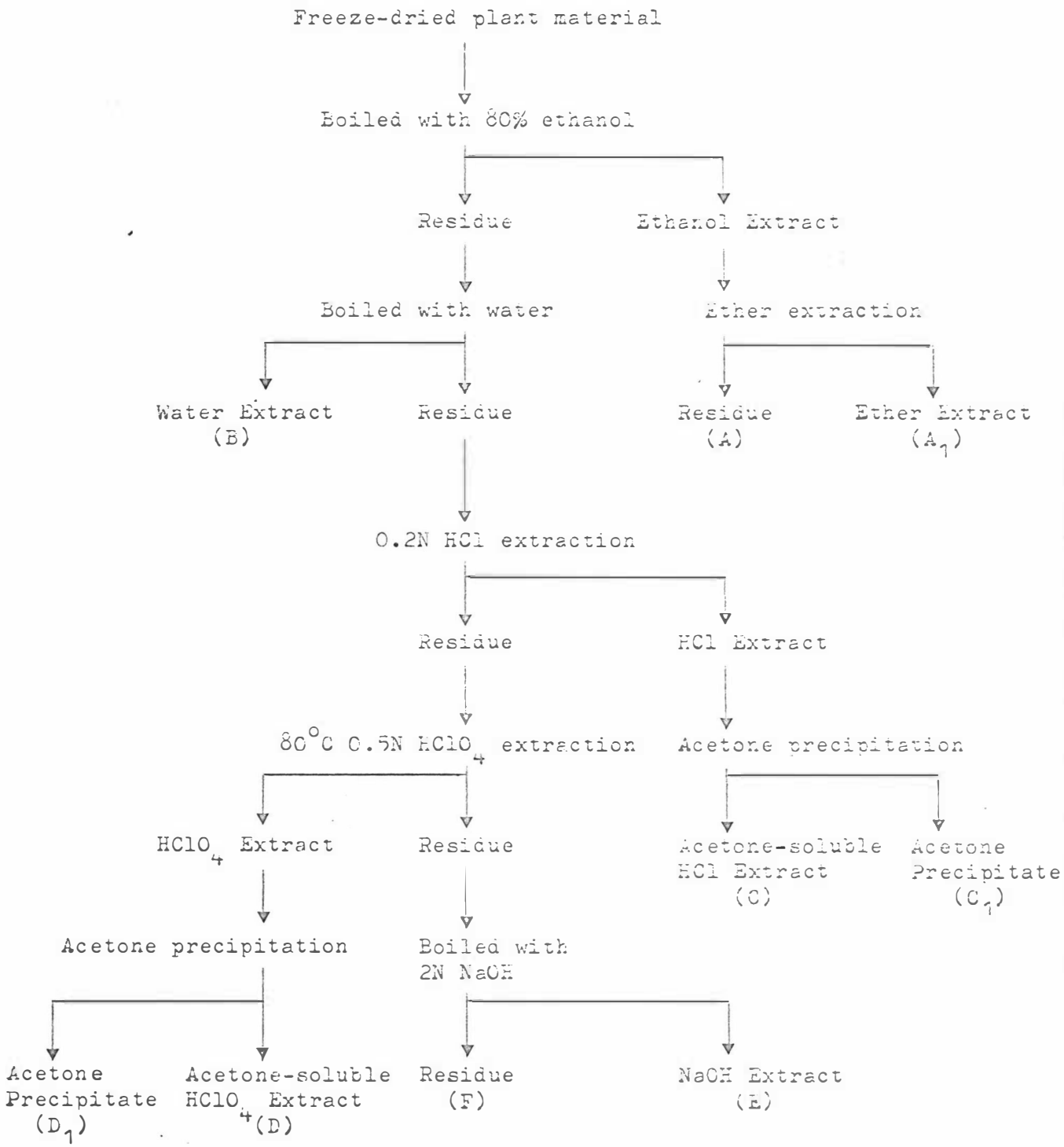

Fig. IV - 5. Successive Extraction Sequence

Modified from Bowen et al (1962)

nutrient solution for seven days, after which the roots were washed and excised from the shoots for freeze-drying. This experiment was carried out concurrently with the radioautographic study.

The roots and shoots of these plants from two different feeding treatments, were then subjected to the above fractionation system, the results being shown in Table IV-2.

These results showed that in particular, three fractions, A_1, C_1 and D_1 contained very small proportions of the radioactivity. This indicated that no significant amount of ^{51}Cr was firmly bound to either the pigments, pectates or nucleic acids. However, 30-50% of the ^{51}Cr was extracted by ethanol and water and was probably as small molecules. This is fortunate, since the soluble fraction is the simplest to study further. Also, especially in the shoots, another large amount of the radioactivity was extracted by hydrochloric acid, probably as polar molecules or ions.

Although there were no significant differences between the plants fed $^{51}CrO_4^{2-}$ and those fed $^{51}Cr^{3+}$, there were greater differences between the fractionation patterns for the shoots and roots, these differences being similar for both those fed CrO_4^{2-} and those fed Cr^{3+}. In particular there was a much larger proportion of water-soluble ^{51}Cr from the roots than from the shoots. However, this pattern was reversed in the hydrochloric acid fraction (C), so that when the ethanol, water and hydrochloric acid fractions were combined, the sum was between 54 and 63%, i.e. all samples had similar total readily soluble fractions. Extraction by perchloric acid also released more ^{51}Cr from shoots than from roots, including more significant portions in the nucleic acid fraction (D_1).

Table IV - 2

CHEMICAL FRACTIONATION OF CHROMIUM-51 IN CLOVERS

(Percentage of counts recovered)

Fraction / Solvent		A Ethanol	A_1	B Water	C HCl	C_1	D $HClO_4$	D_1	E NaOH	F
Cr^{3+} fed	Shoots	18.7	0.1*	24.3	20.7	0.4	16.6	1.7	12.9	4.6
	Roots	19.7	n.d.	30.1	6.9	1.9	4.8	0.33	23.2	13.1
CrO_4^{2-} fed	Shoots	15.3	0.3*	15.3	24.3	0.6*	13.8	5.2	19.6	5.5
	Roots	16.5	n.d.	36.3	11.4	2.6	8.6	0.1*	17.4	7.1

* Activity not significantly above background

n.d. Not determined.

The caustic soda fraction (E) contained appreciable amounts (12-24%) of ^{51}Cr suggesting that this was from degraded proteins or polysaccharides. However, little radioactivity remained in the residue, with the exception of the roots from the plants fed $^{51}Cr^{3+}$.

These results can be compared with the work of Bowen et al (1962) who studied eleven radioisotopes in tomato leaves. Except for 40% of the ^{45}Ca in pectates, not much radioactivity was found in nucleic acids, proteins or structural portions of the plant. For most elements, 70% of the activity was extracted by ethanol and hydrochloric acid, although they did not include a water extraction.

In view of the work of Bourque et al (1967) which suggested that ^{51}Cr was predominantly bound to the protein fraction, preparations were made of soluble proteins and ribonucleic acid (RNA) directly from the active tissue rather than by the successive extraction method of Bowen et al (1962). Samples of the plant material from the uptake experiments were ground and homogenised in 0.05 M tris-HCl buffer at pH 7.5 (tris = 2-amino-2-hydroxymethylpropane-1,3-diol), and the debris spun down. The supernatant was separated into two equal volumes.

One half of this solution was used to separate RNA by the method of Moustafa and Lyttleton (1963) which required the addition of 0.8 volumes of water-saturated phenol, blending at $1^{o}C$, followed by separation by centrifugation. The RNA was precipitated from the upper layer by the addition of two volumes of ethanol at $1^{o}C$, and separated by further centrifugation. The other half of the solution was used to isolate soluble proteins by precipitation with trichloracetic acid (TCA). TCA was added to the buffer making the solution 5% w/w to precipitate proteins, which were spun down.

This precipitate was resuspended for washing, centrifuged, resuspended again in 5% TCA solution, heated for 30 minutes at 90°C, cooled and spun again. This last precipitate was collected as proteins.

The results of counting these preparations are presented in Table IV-3. Examination of the table shows for all tissues that, although a large amount of the radioactivity was soluble in tris buffer, the proteins contained less than 0.25% of the total activity. These results are quite different from the report of Bourque et al (1967).

(v) The Chemical Form of Chromium-51 in Clover

High voltage electrophoresis of standard $^{51}CrCl_3$ and $Na_2{}^{51}CrO_4$ solutions showed that, as expected, these two forms are readily separated. This could be shown either by radioautography or by scanning the electrophoretogram with the scintillation detector. Figure IV-6 shows the electrophoretic patterns at pH 5.3. The sodium ^{51}Cr-chromate solution shows a predominant peak of the $^{51}CrO_4{}^{2-}$ ion, but the ^{51}Cr-chromic chloride solution shows five pronounced peaks of radioactivity. In order of increasing mobility on electrophoresis these probably correspond to the species: $[Cr(H_2O)_3Cl_3]^0$, various isomers; trans-$[Cr(H_2O)_4Cl_2]^+$; cis-$[Cr(H_2O)_4Cl_2]^+$; $[Cr(H_2O)_5Cl]^{2+}$; $[Cr(H_2O)_6]^{3+}$.

Clovers, which had been fed ^{51}Cr for 10 hours, and then returned to nutrient solution for 5 days before harvesting, were extracted with 80% ethanol as for the first stage of the fractionation experiments. These extracts were concentrated in a rotary film evaporator at less than 40°C. Samples were subjected to electrophoresis at pH 5.3, in pyridine-acetic acid buffer.

Table IV - 3

CHROMIUM-51 RADIOACTIVITY IN PROTEIN AND RNA FROM CLOVER

(Percentage of total activity)

		Soluble at pH 7.5	RNA	Protein
Cr^{3+} fed	Shoots	45.9	2.6	0.18
	Roots	37.7	9.9	0.20
CrO_4^{2-} fed	Shoots	50.6	6.9	0.19
	Roots	27.3	6.5	0.22

Percentage of original activity

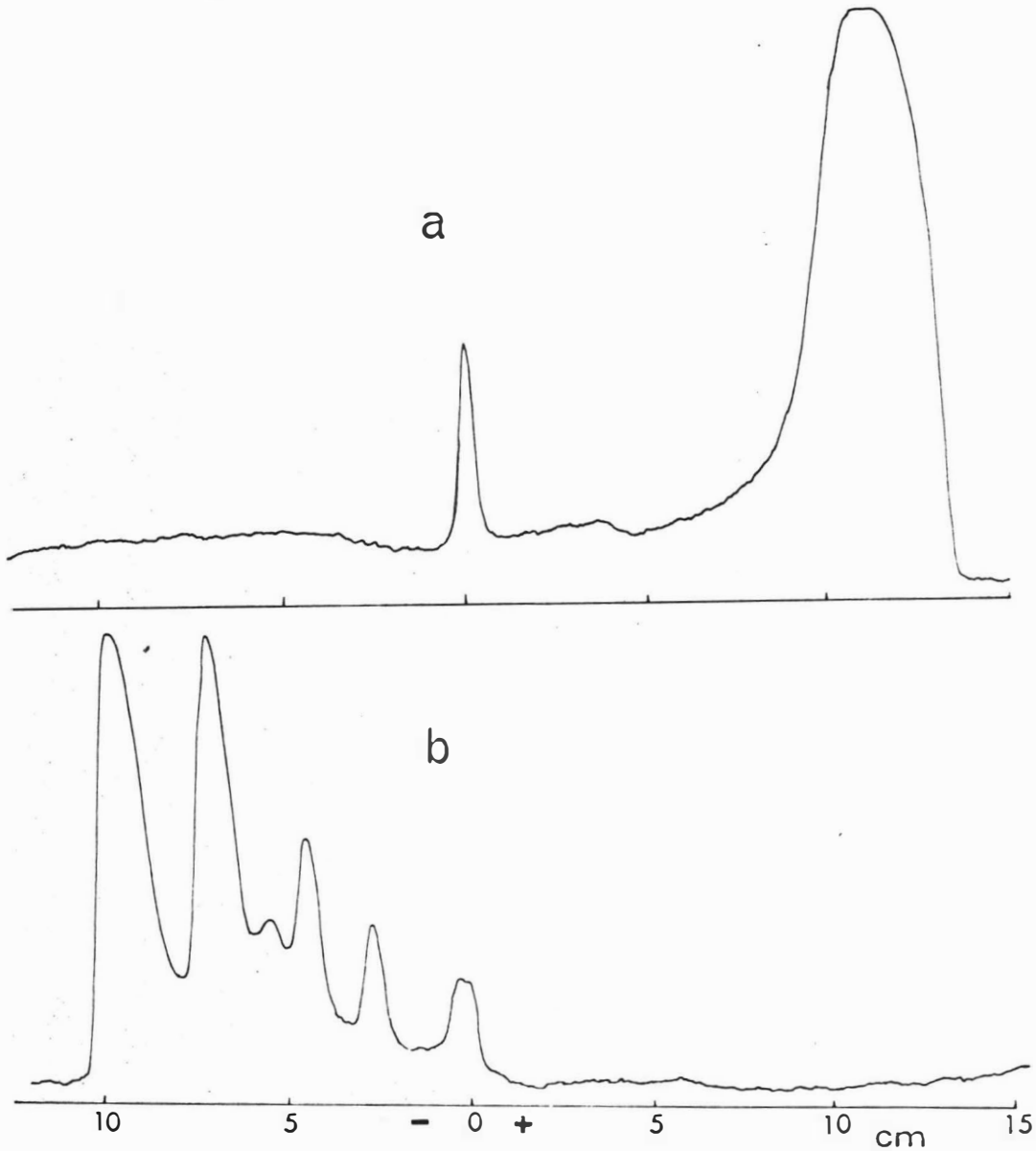

Fig. IV - 6. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis.

a) Standard CrO_4^{2-}.

b) Standard Cr^{3+}.

Figure IV-7 shows the radioactivity distribution of some of these ethanol extracts. Other extracts did not contain sufficient radioactivity to obtain a meaningful scan pattern. The ethanol extracts of clover roots, both those fed Cr^{3+} and those fed CrO_4^{2-}, contained compounds which were anionic at pH 5.3, but were not the same as chromate, although it appeared that chromate was also present in relatively small amounts.

The major component, here called compound B, moved at a slower rate than chromate, and another peak, compound A, occurred in some electrophoretic patterns. Compound A, which was nearer the origin than compound B, was invariably at a lower level of activity than was B. Neither of these compounds is as yet identified, although both appeared to be also present in manuka (see later).

A further experiment used clovers (40 days old) which were fed $^{51}CrO_4^{2-}$ for 48 hours before being harvested. 750 µCi ^{51}Cr in 30 ml of 0.2 ppm chromium solution was supplied to five plants. The leaves were separated from the petioles, and each of the leaves, petioles, and roots were extracted with boiling 80% ethanol. These extracts were concentrated, the leaf and petiole extracts were dissolved in water and ether and the ether layer discarded, as this had been shown to contain negligible amounts of ^{51}Cr (Table IV-2). The concentrates were then electrophoresed at pH 5.3 and Figure IV-8 shows scans of the radioautograph patterns of these electrophoretograms. These patterns show that, although the different parts of the plants contained different amounts of activity, compounds A and B were both present in each part.

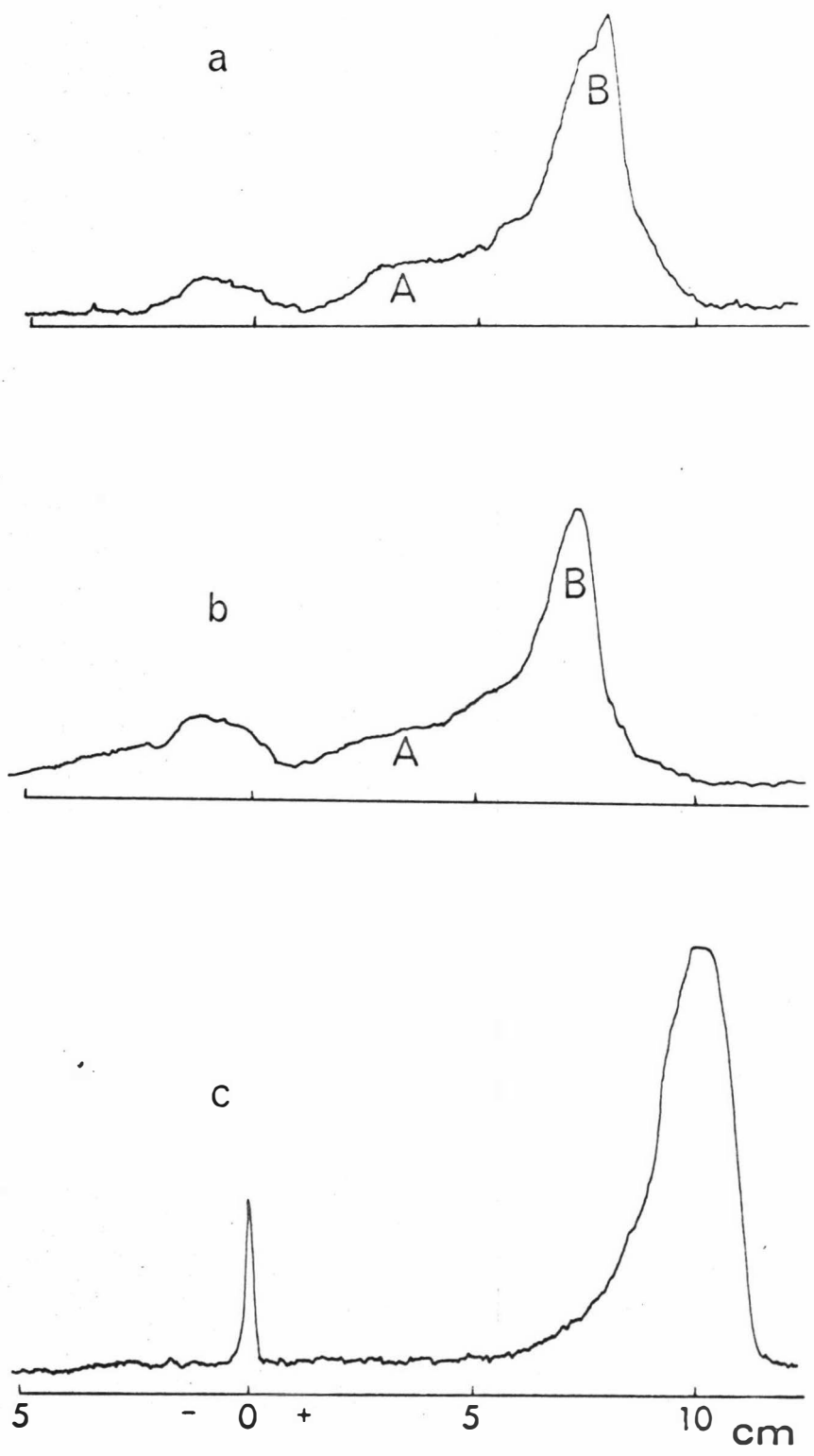

Figure IV - 7

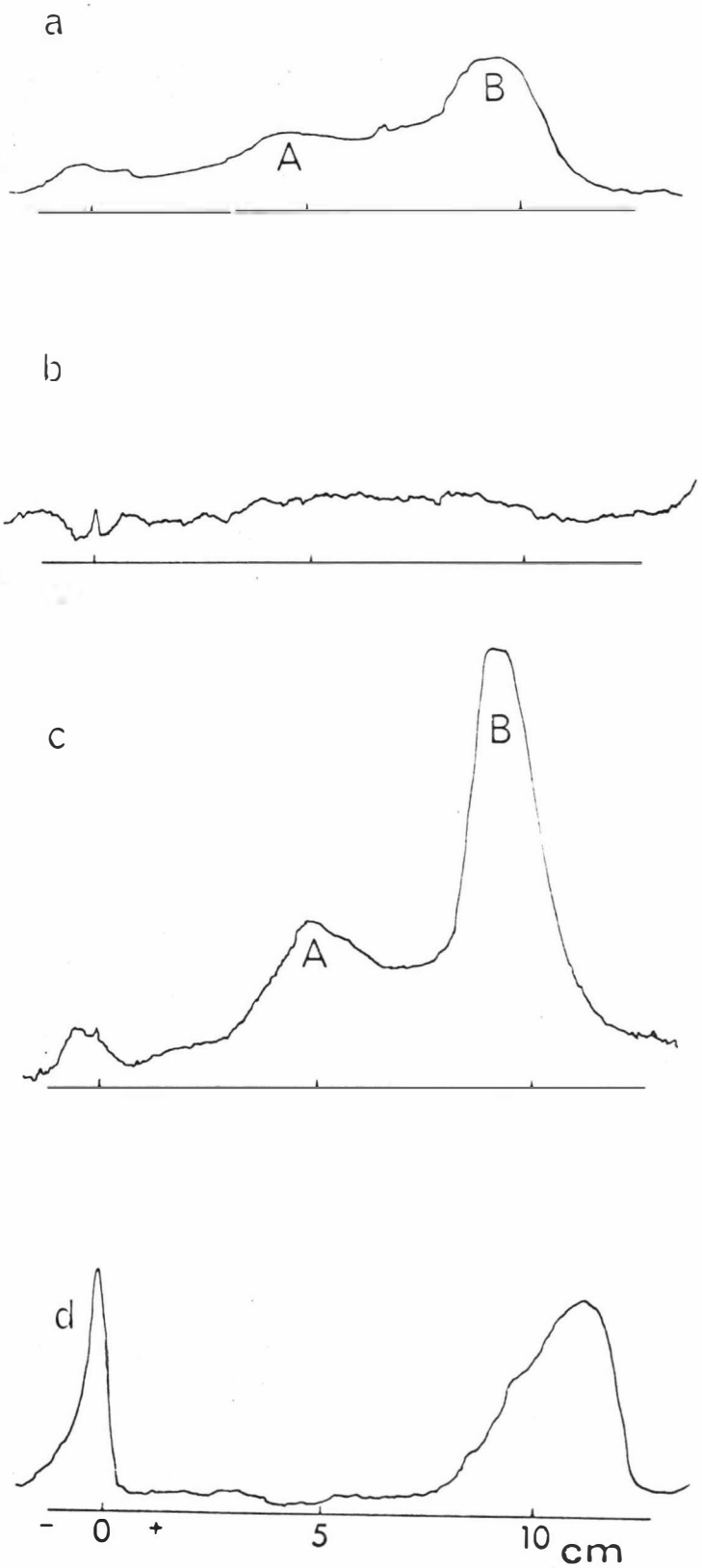

Figure IV - 8.

4. EXPERIMENTS WITH PLANTS FROM THE MINERAL BELT

In May 1966, several small plants of the species studied in Part III, together with a quantity of soil, were collected from near the Dun Mountain Tramway (Site 15, Figure III-2), and were brought to Palmerston North. All samples of the species endemic to the Mineral Belt were then potted into serpentine soil, but Notothlaspi australe and Myosotis monroi plants failed to adapt. However, Pimelea suteri, a woody plant, was able to continue growing. Half of the samples of the other three species, Hebe odora, Cassinia vauvilliersii and the manuka Leptospermum scoparium were also potted into serpentine soil. The other samples were potted into a soil consisting of four parts of Manawatu silt-loam to one part of pumice. All these plants were kept in a glass-house and watered as required.

After a year, there were some quite noticeable differences between the growth of plants potted in different soils. Those plants that were in the Manawatu soil had grown much more than had the others in serpentine soil, where often no increase in size was visible. This difference was most noticeable for the manuka, which in serpentine had not grown appreciably, although with the plants in Manawatu soil some branches had grown by more than 10 cm.

However, with a limited number of these specimens available for experimentation, only general trends and observations can be considered significant in the translocation experiments.

(i) Translocation Experiments

Several plants while growing in the glass-house, were studied for evidence of translocation of ^{51}Cr.

Two methods of feeding were used:

(a) Some plants (referred to later as "stem fed") had a small cut made on the stem of the plant to remove bark and expose the xylem. To this cut 1 μl of sodium chromate solution containing 0.007 μg Cr and about 1 μCi ^{51}Cr was applied daily with a glass micropipette.

(b) The other plants were "tip fed". For H. odora, this involved puncturing the terminal leaf bud and injecting into it 1 μl of ^{51}Cr solution. For the other species, manuka (L. scoparium), C. vauvilliersii and P. suteri, the apical tip was cut off (under water to prevent drying of the vessels) and this tip was then dipped into a 1 ml beaker containing 10 μl of $^{51}CrO_4^{2-}$ solution and 1 ml of distilled water. This solution was renewed daily. During a period of hot weather, it was found necessary to replenish these beakers with water in the morning in addition to replacing the solution every afternoon. At these times too, water was also applied to the cut surfaces of the other plants in an attempt to prevent excessive surface drying.

These plants, after 16 months in the glass-house, were fed ^{51}Cr daily for 14 days. Then, 19 days after the initial feeding each plant was cut at the soil surface, the roots were washed free of soil and the whole plant material was freeze-dried. Woody plants were cut into sections, care being taken to keep the pieces in their correct relative positions.

After drying, these plants were pressed and glued on to paper and then radioautographed for 8 days. The radioautographs of those plants which showed movement of ^{51}Cr are shown in Figures IV-9 to IV-16, together with photographs of the dried specimens. In some

cases, detail has been lost during reproduction. Arrows show the point of application of the ^{51}Cr solution. The results are discussed for each species.

(a) Cassinia vauvilliersii

Of the four samples from serpentine soil, two were tip fed and two were stem fed, but no activity was observed other than at the point of application. Four similar plants which had been growing on Manawatu soil were similarly fed. One of those which was tip fed, showed no movement but the other showed slight activity spreading down about 4 cm from the tip. The two plants which were stem fed, however, showed activity in branches above the point of application, as in Figure IV-9. No activity was observed in any roots. The radioautograph suggests that ^{51}Cr, when applied to a cut in the stem, is not moved around the stem but ascends only on the same side of the stem as the cut, and into the branches fed by these xylem vessels. Also it appears that ^{51}Cr is not readily moved from the tip downwards.

(b) Hebe odora

As with the C. vauvilliersii, four plants were taken from each soil type, and two of each were tip fed and two were stem fed. For only two plants was there activity visible other than at the point of application. A tip-fed plant (Figure IV-10) from serpentine soil showed that some of the apical leaves had ^{51}Cr activity especially at the edges. During the course of the treatment, the apical bud which was being fed, had opened, and the active leaves may be merely those which had been in contact with the feeding solution. The other radioautograph (Figure IV-11) is of a plant from Manawatu soil, which was stem fed, like the C. vauvilliersii (Figure IV-9) indicating

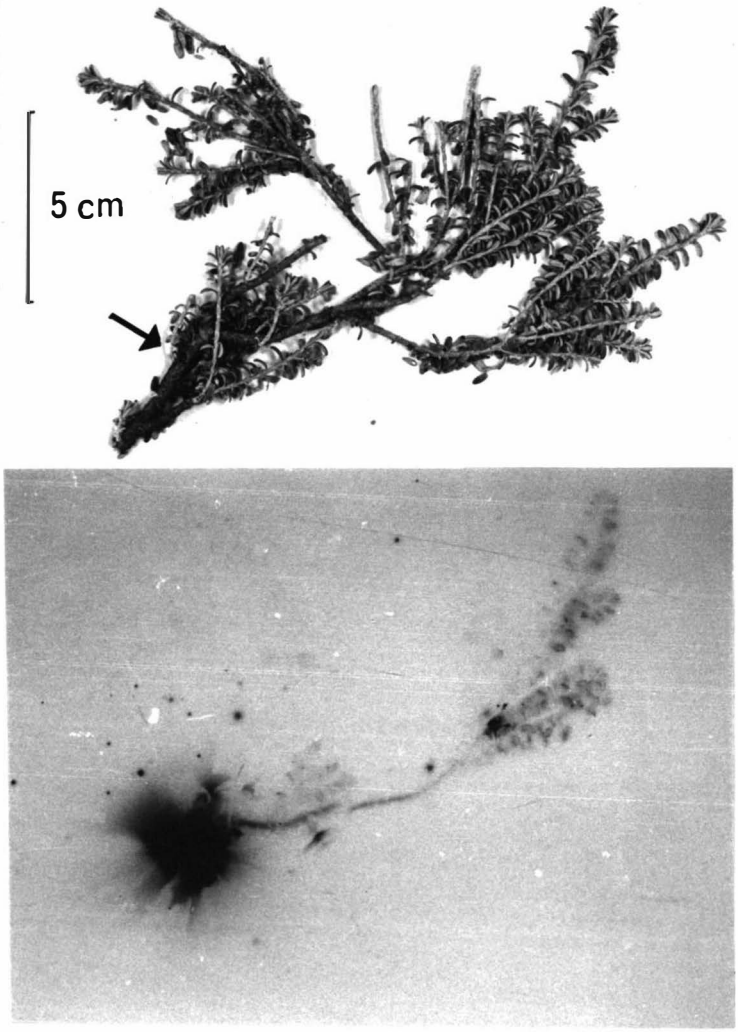

Fig. IV - 9. Cassinia vauvilliersii from Manawatu soil.
^{51}Cr applied to the stem.
Upper : photograph. Lower : radioautograph.

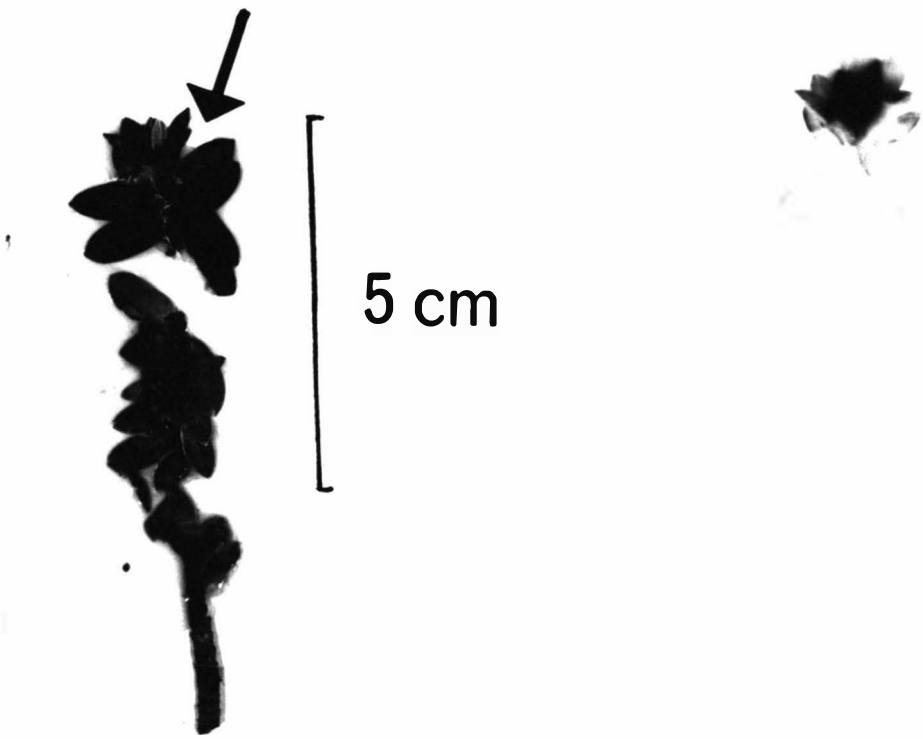

Fig. IV - 10. Hebe odora from serpentine soil.
^{51}Cr injected into the leaf bud.
Left : photograph. Right : radioautograph.

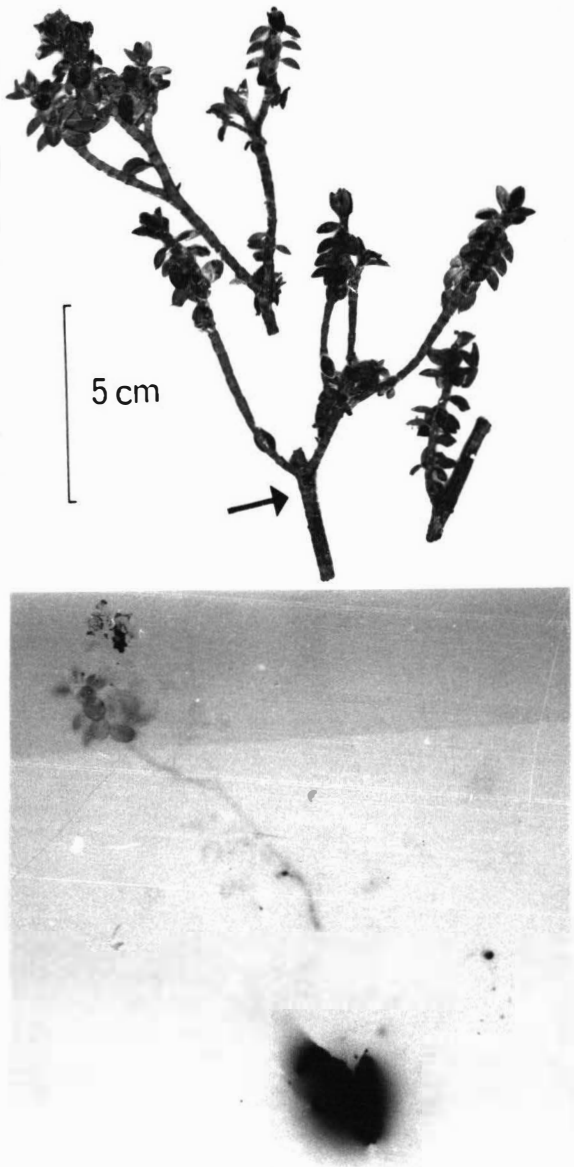

Fig. IV - 11. Hebe odora from Manawatu soil.
^{51}Cr applied to the stem.
Upper : photograph. Lower : radioautograph.

movement of ^{51}Cr up the stem on the side of the plant where the radioisotope was applied. Again no activity was observed in any roots.

(c) Leptospermum scoparium (manuka)

Two plants from serpentine soil were tip fed and radioautographs showed that ^{51}Cr was able to move only a few centimetres down the fed stem, in the case of a single-stem plant, or down a branch and up other branches. Two other plants from serpentine soil were fed ^{51}Cr at a cut stem and both showed slight movement of the activity up the stems as in Figure IV-12. Of those which had been growing in Manawatu soil, one of the tip fed specimens showed activity in most leaves from the fed stem-tip to its base, as shown in the radioautograph in Figure IV-13, although the other showed activity not more than 4 cm from the point of application. The one sample from this soil which was stem fed, showed movement of radioactive material up the stem and into the branches on the side of the stem above the cut (Figure IV-14).

There were considerable differences in the morphology of these manuka plants because some of these were in Manawatu soil for over a year allowing a more normal rate of growth, while others continued a relatively stunted rate in serpentine soil. Also some of these plants were at some stages infected with manuka blight (Eriococcus orariensis Hoy) which spraying did not completely eradicate. However, in general there does not appear to be any major difference in the patterns of activity between the manukas growing in serpentine soil and those in Manawatu soil.

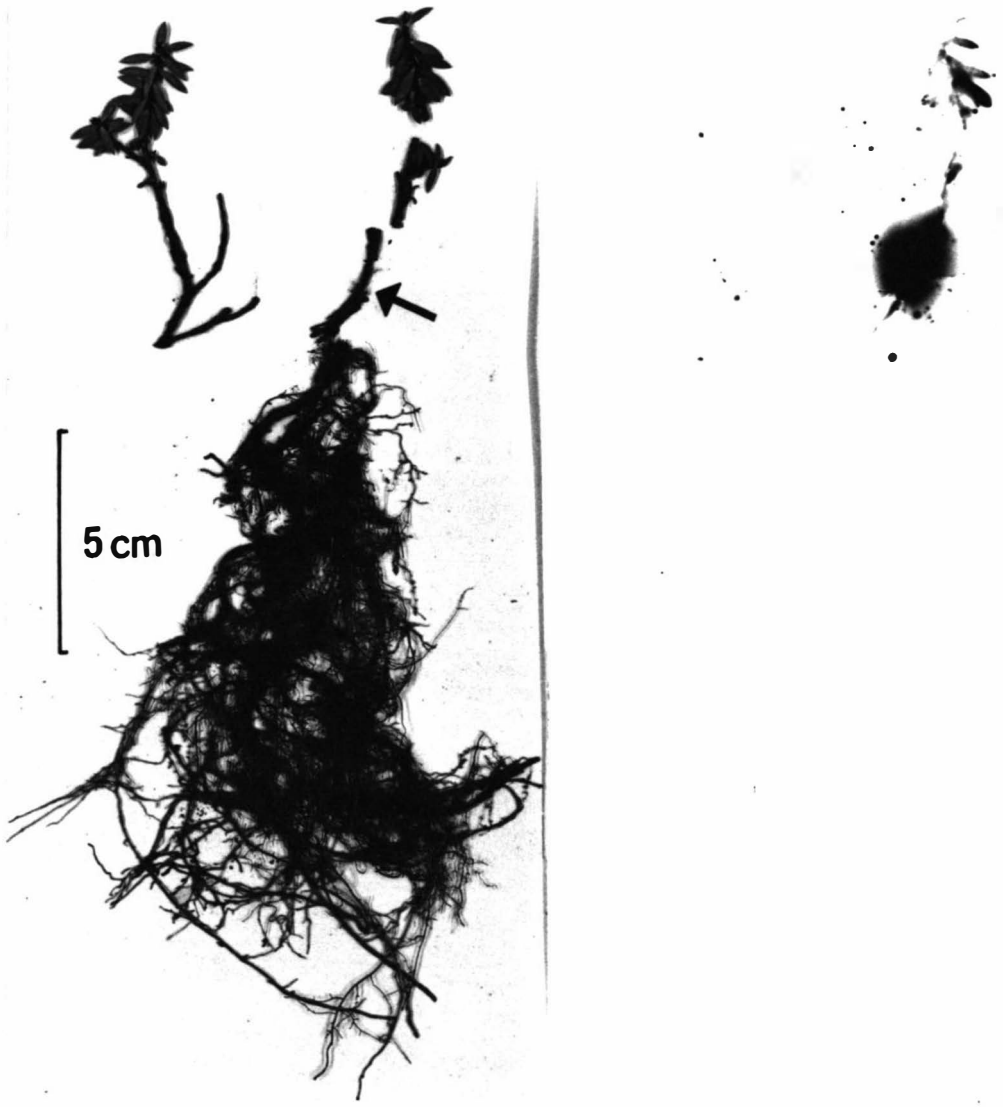

Fig. IV - 12. Leptospermum scoparium from serpentine soil.
^{51}Cr applied to the stem.
Left : photograph. Right : radioautograph.

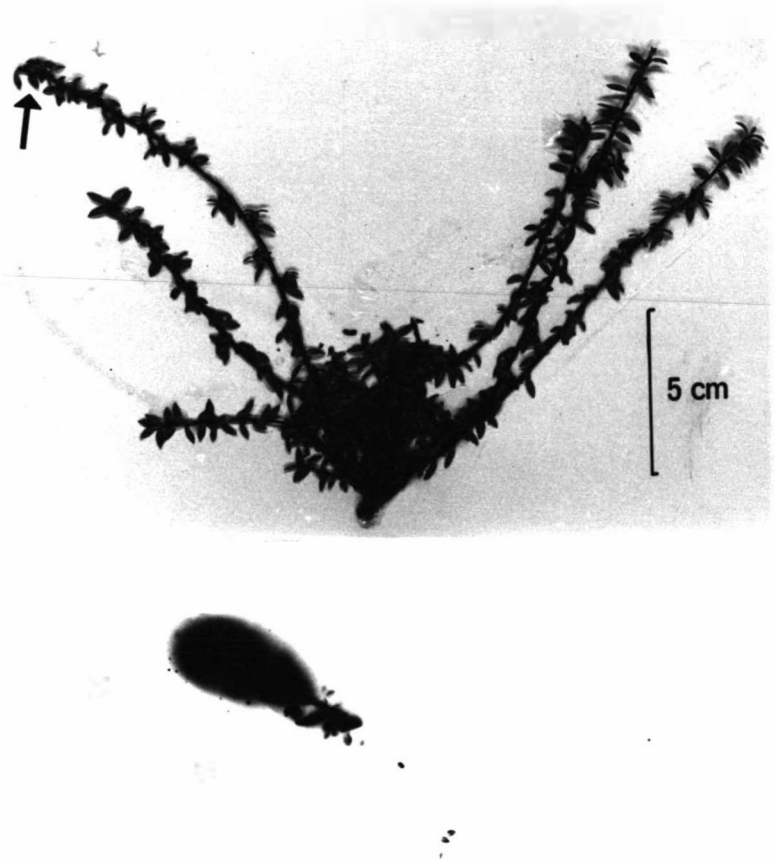

Fig. IV - 13. Leptospermum scoparium from Manawatu soil.
Tip of a branch dipped into ^{51}Cr solution.
Upper : photograph. Lower : radioautograph.

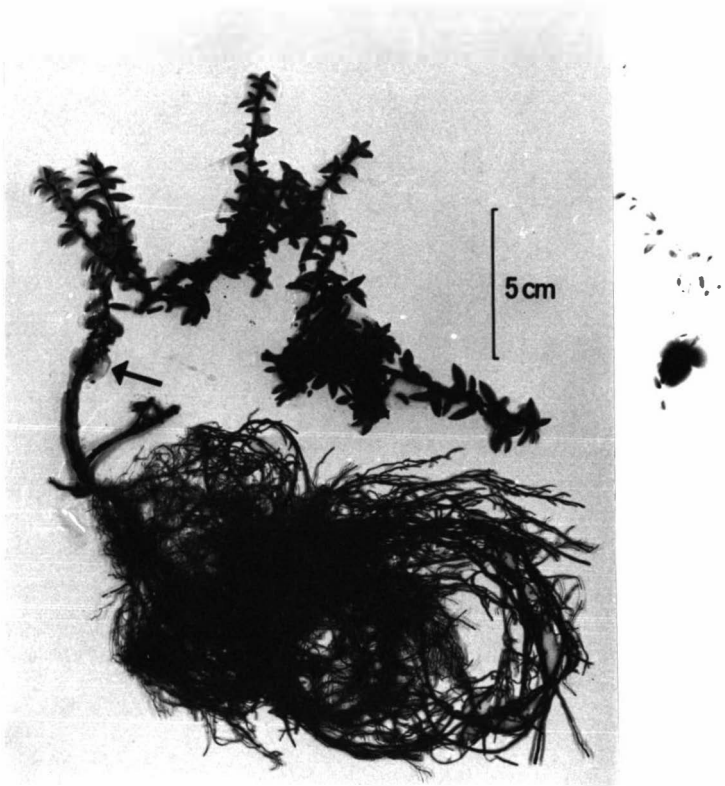

Fig. IV - 14. Leptospermum scoparium from Manawatu soil.
^{51}Cr applied to the stem.
Left : photograph. Right : radioautograph.

(d) Pimelea suteri

All of these small shrubs were grown on serpentine soil to which they are endemic (Betts 1918, C.J. Burrows pers. comm.). One plant (Figure IV-15), whose lower branch dipped into ^{51}Cr solution, showed movement of radioactive chromium into this branch and into and up the main stem of the plant. There appeared to be more activity in the leaves than in the stems, and ^{51}Cr accumulated at the tips and edges of the leaves. A similar plant fed at the tip of a longer shoot also showed movement back down its stem and into other branches and again ^{51}Cr was concentrated at the tips of the leaves. The third specimen (Figure IV-16) was stem fed and showed more activity away from the point of application than did any other plant of the four species studied. Radioactivity was spread throughout most of the plant and again concentrated in the tips of leaves. The radioautograph also shows that most movement had occurred on the side of the stem where the cut was made.

(e) Discussion

In general therefore, manuka (L. scoparium) and P. suteri appear to be able to move ^{51}Cr more readily than C. vauvilliersii or H. odora. This is consistent with the analytical data in Part III which showed that P. suteri and manuka have a much higher average chromium content and a higher plant:soil ratio for chromium than do the other two species. In all these plants however, there is the possibility of immobilisation by absorption or precipitation at the site of application, either by plant material or soil particles on the surface of the plants. If this occurs, then non-movement of ^{51}Cr is not an indication that chromium cannot move in the plant.

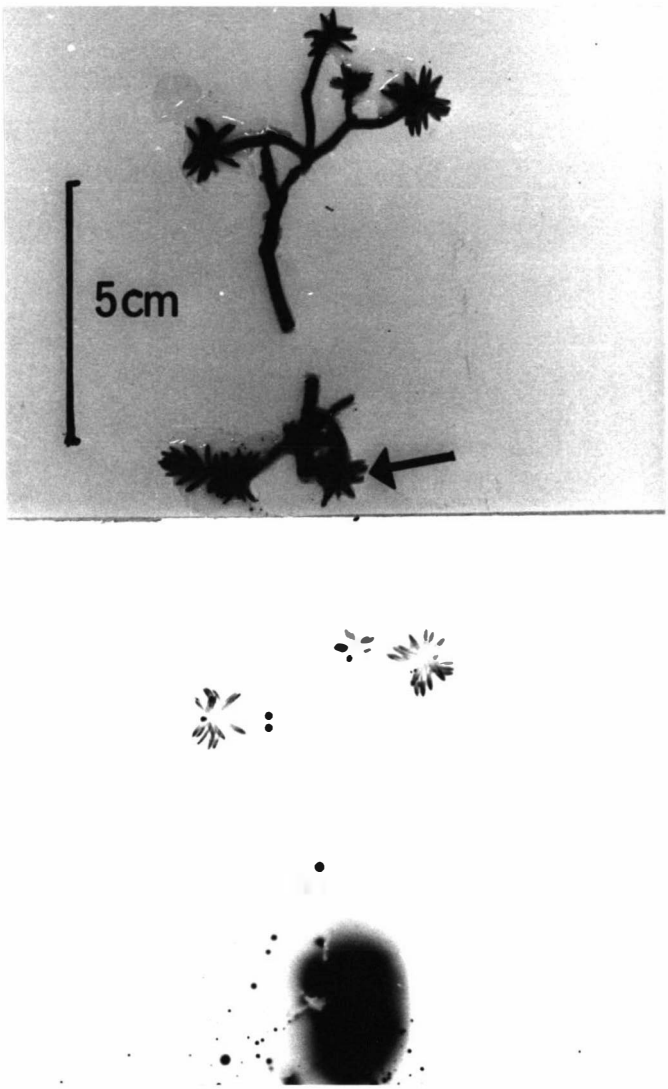

Fig. IV - 15. Pimelea suteri from serpentine soil.
The tip of a lower branch was dipped into ^{51}Cr solution.
Upper : photograph. Lower : radioautograph.

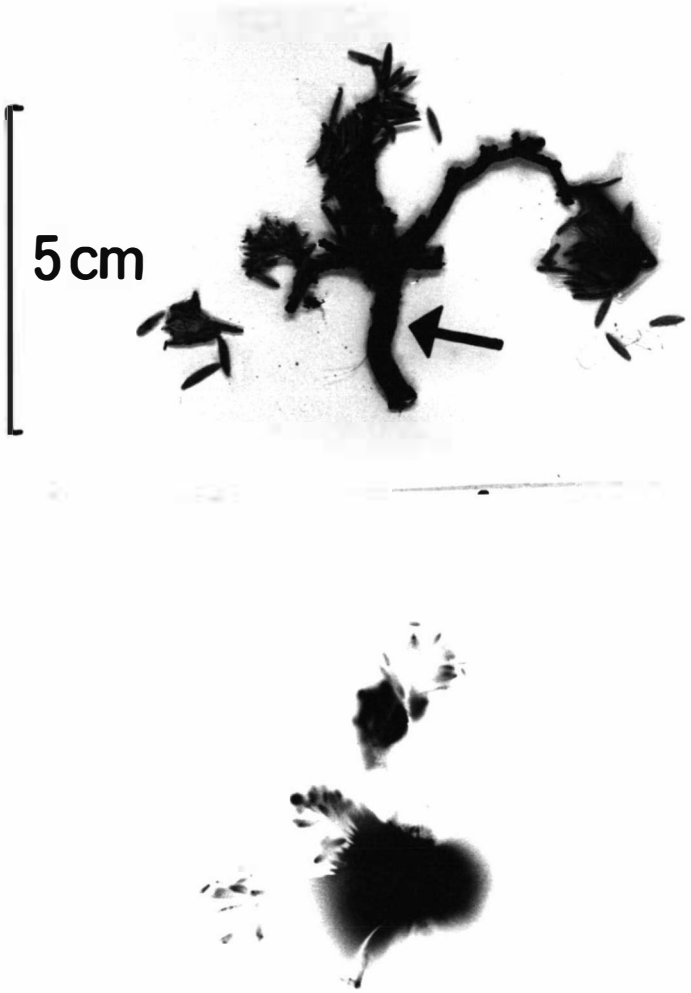

Fig. IV - 16. *Pimelea suteri* from serpentine soil.
^{51}Cr applied to the stem.
Upper : photograph. Lower : radioautograph.

(ii) Chemical Studies

These experiments were carried out only on H. odora and L. scoparium, as no suitable specimens of the other species were available.

(a) Hebe odora

Four of the plants brought back from the Mineral Belt 16 months previously, and potted in serpentine soil had the soil washed from their roots and were transferred into a full strength nutrient solution in the growth cabinet. Four plants which had been potted in Manawatu soil were treated in a similar way, but one of these did not survive the transfer to solution culture.

The seven surviving plants were in nutrient solution culture for 16 days, and were then washed and transferred into a solution containing 0.03 ppm chromium as sodium ^{51}Cr-chromate (400 μCi for each of the two groups of plants). After 24 hours in this solution, the plants were harvested and freeze-dried.

Table IV - 4

CHROMIUM-51 RADIOACTIVITY IN HEBE ODORA

(c/m/mg of dry plant)

Soil type	Leaves	Stems	Roots
Serpentine	0.084	10.5	789
Manawatu	0.32	0.27	510

Leaves, stems and roots were all powdered separately. Individual plant variation was not studied but Table IV-4 shows quite significant differences between the ratios of ^{51}Cr activity in the roots, stems and leaves for plants from different soils. These results suggest that the plants growing on serpentine soil were more readily able to transport ^{51}Cr to the stems from the roots but found it more difficult to move ^{51}Cr on to the leaves than did the plants grown in Manawatu soil.

Because of the very low activities of the leaves of both types of H. odora and of the stems of the plants grown on Manawatu soil, these samples were not studied further. The other samples were chemically fractionated using the modification of the method of Bowen et al (1962) in the same manner as the clovers earlier. The results of this are shown in Table IV-5. As before, these results are recalculated on the basis of counts recovered, whereas the actual recoveries were as follows:

Roots from plants in Manawatu soil 94.5%

Roots from plants in serpentine soil 83.0%

Stems from plants in serpentine soil 73.3%

The low recoveries cannot be accounted for by statistical variation, but indicate losses during fractionation.

In general the most obvious information from Table IV-5 is that most of the radioactivity is removed only in the later stages of extraction. This may, at least in part, be due to some of the activity having been absorbed by soil particles. The stems, however, show an unusually high proportion of the radioactivity soluble in the water fraction.

Table IV - 5

CHEMICAL FRACTIONATION OF CHROMIUM-51 IN HEBE ODORA

(Percentage of counts recovered)

Fraction / Solvent		A Ethanol	A_1	B Water	C HCl	C_1	D $HClO_4$	D_1	E NaOH	F
Soil Type	Part of Plant									
Serpentine	Stems	11.5	0.1*	38.4	2.9	1.3	14.7	0.1*	16.8	14.2
Serpentine	Roots	12.3	n.d.	13.1	6.8	0.1*	19.2	0.5	30.0	18.0
Manawatu	Roots	4.8	n.d.	9.7	11.3	0.7	20.9	0.6	37.9	14.1

* Activity not significantly above background

n.d. Not determined

Protein and RNA fractions were also prepared as before, and only the stem protein fraction contained any appreciable quantities of ^{51}Cr (Table IV-6).

Table IV - 6

CHROMIUM-51 RADIOACTIVITY IN PROTEIN AND RNA FROM HEBE ODORA

(Percentage of total activity)

Soil type	Part of Plant	RNA	Protein
Serpentine	Stems	0.5*	6.8
Serpentine	Roots	0.05*	0.2
Manawatu	Roots	0.01*	0.01*

* Not significantly above background

Radioautography of two of these plants showed no differences between the roots which had been in Manawatu soil, and those which had always been in serpentine soil.

Electrophoresis at pH 5.3 was carried out with the ethanol and water extracts from the successive extraction separation. These patterns were not radioautographed but the paper strips were scanned using the crystal scintillation head. Because of inefficient collimation of the γ-rays, resolution on these scans (Figure IV-17) was far poorer than densitometer scanning of radioautographs. Some of these patterns did not show any significant concentrations

Fig. IV - 17. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis.

Extracts of soil-grown Hebe odora fed with $^{51}CrO_4^{2-}$.

(a) Plant grown in serpentine soil : ethanol extract of roots.

(b) Plant grown in serpentine soil : water extract of roots.

(c) Standard $^{51}CrO_4^{2-}$.

(d) Plant grown in Manawatu soil : ethanol extract of roots.

(e) Plant grown in Manawatu soil : water extract of roots.

(f) Plant grown in serpentine soil : ethanol extract of stem.

(g) Plant grown in serpentine soil : water extract of stem.

(h) Standard $^{51}CrO_4^{2-}$.

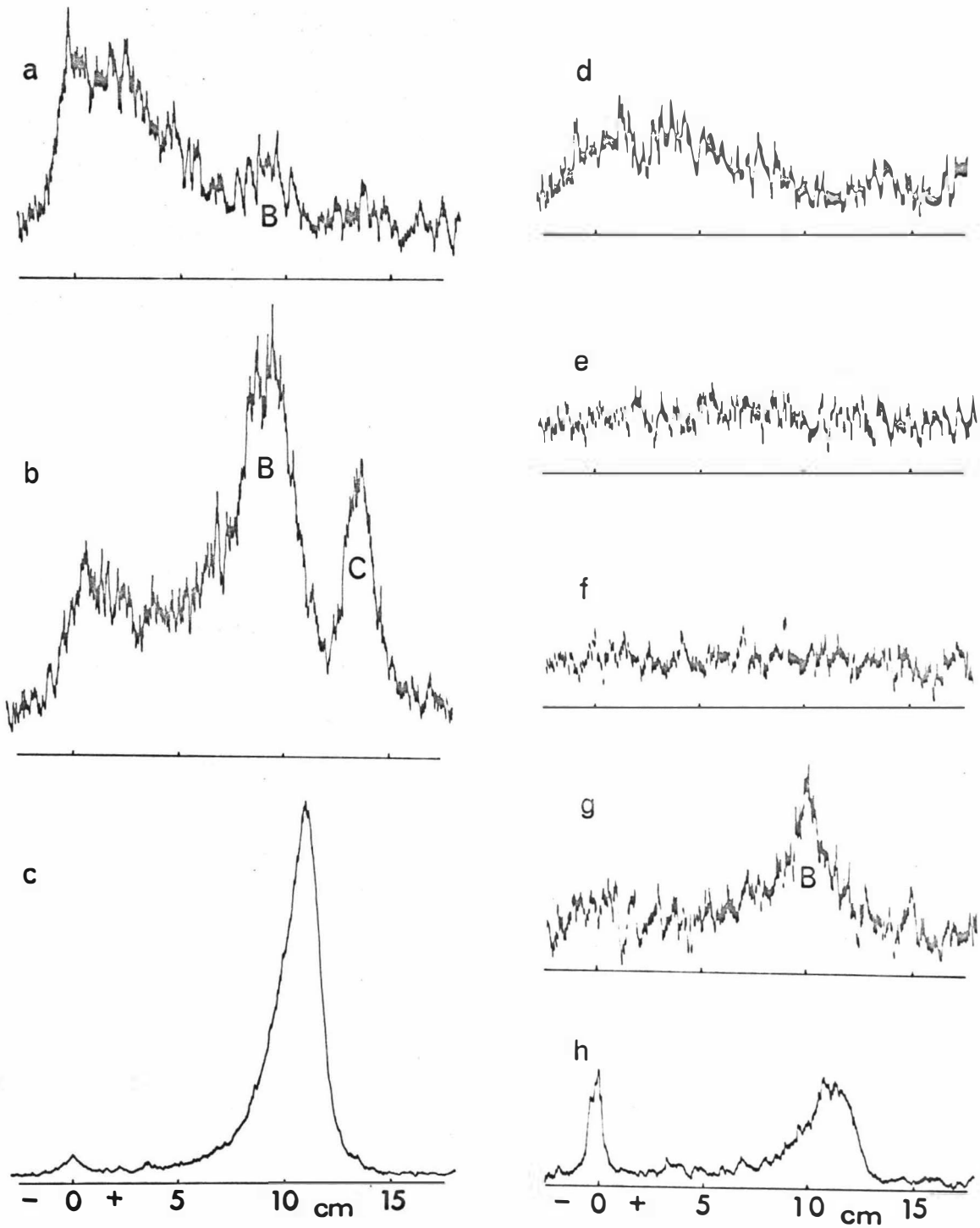

Figure IV - 17.

of ^{51}Cr, but others all showed some of the activity at the origin and the rest as anions.

A peak, labelled compound B, but which may be chromate, was present in the water extract from stems of plants which had been growing in serpentine soil, and also occurred in the water and ethanol extracts of the same plants. In addition, the water extract of these roots showed a fast anionic peak, compound C, which appeared to be the trioxalatochromate(III) complex (see later).

Partial analyses of the roots of the plants from these two soil types are given in Appendix 3.

(b) Leptospermum scoparium (manuka)

After 18 months in a glass house in Palmerston North, six plants from serpentine soil and three from Manawatu soil were removed from their pots and the soil was washed from their roots. Experience had shown that these manukas were not able to survive for very long if transferred into a nutrient solution in the growth cabinet. As a result, these plants were placed directly into a solution containing ^{51}Cr.

This solution contained 0.005 M $Ca(NO_3)_2$ and 0.03 ppm total chromium as $Na_2{}^{51}CrO_4$ (150 μCi per three plants). After 48 hours in this solution in the growth cabinet, the plant roots were again washed, and the plants harvested and freeze-dried. When dry, the leaves were separated from the stems and specific activities were determined separately for leaves, stems and roots.

The results (Table IV-7) showed differences in the distribution of radioactivity between the plants grown in serpentine soil and those grown in Manawatu soil. There also appeared to be a significant

Table IV - 7

CHROMIUM-51 RADIOACTIVITY IN MANUKA FROM SOIL

Soil Type	Sample No.	Activity (c/m/mg)			Dry weight (mg)		
		Leaves	Stems	Roots	Leaves	Stems	Roots
Serpentine							
Group (a)	S1	4.25	67.67	1659	146	68.0	224
	S3	3.31	39.92	1522	226	95.6	479
	S6	4.03	35.28	1504	86.7	89.0	193
Group (b)	S2	3.82	4.26	1695	128	41.5	276
	S4	4.12	5.96	1279	164	54.4	463
	S5	2.99	6.55	1540	173	88.8	487
Manawatu							
	M1	2.76	1.46	599	753	417	985
	M2	1.83	0.98	594	627	478	1025
	M3	2.27	4.66	639	887	786	1128

difference among the plants from serpentine soil, allowing these to be divided into two groups. These had leaf : stem : root specific activity ratios as follows:

Serpentine soil Plants Group (a) - 4 : 35-70 : 1500 c/m/mg

" " " " (b) - 3 : 4-7 : 1500 c/m/mg

Manawatu soil Plants - 2 : 1-5 : 600 c/m/mg

Since the plants which had been on Manawatu soil for 18 months were much larger than those from serpentine soil, this may account for the different radioactivity ratios between these plants, but size alone cannot be used to explain the differences between the two groups of serpentine-soil plants.

To obtain sufficient radioactivity for chemical fractionation, samples were combined within the three divisions above. Table IV-8 shows the results of chemical extraction by the modified Bowen method, as for the clovers. Considering the leaves, there was little difference between the two serpentine plant groups. However the ^{51}Cr in leaves from the plants from Manawatu soil was less soluble in boiling water (fraction B) but more so in hydrochloric acid (C) and perchloric acid (D) than in leaves from plants from serpentine soil. Chromium-51 from the stems of the serpentine group (a) plants (those with a relatively high stem activity) was less soluble in boiling water (fraction B) than ^{51}Cr from the stems of the serpentine group (b) plants, with the same fraction from the Manawatu soil stems being greater again. Other fractions-however were similar for all samples. Comparison with Table IV-5 however, shows that very much smaller amounts of ^{51}Cr are soluble in boiling ethanol or boiling water from manuka stems than from the stems of H. odora. With the roots, there were few differences between the extraction patterns for serpentine soil roots and those acclimatised to Manawatu soil.

Table IV - 8

CHEMICAL FRACTIONATION OF CHROMIUM-51 IN MANUKA FROM SOIL

(Percentage of counts recovered)

Fraction		A	A_1	B	C	C_1	D	D_1	E	F
Solvent		Ethanol		Water	HCl		$HClO_4$		NaOH	
Part of Plant	Soil Type									
Leaves	Serpentine (a)	1.4	0.1*	32.3	13.8	0.1*	28.7	1.0	17.4	5.2
	Serpentine (b)	1.6	0.2	37.8	5.9	0.2	30.6	1.3	18.0	4.4
	Manawatu	1.2	0.1*	7.1	29.3	0.1*	47.7	1.2	8.4	5.0
Stems	Serpentine (a)	1.2	n.d.	8.8	10.5	0.2	13.4	1.1	36.2	28.6
	Serpentine (b)	0.8	n.d.	16.2	10.2	0.1*	17.9	3.6	24.7	26.5
	Manawatu	0.5	n.d.	20.7	7.8	0.5*	20.6	0.6	31.7	17.6
Roots	Serpentine	2.7	n.d.	14.8	11.9	0.1*	22.1	0.2	15.8	32.4
	Manawatu	0.9	n.d.	9.2	19.0	0.1*	22.2	0.3	18.9	29.4

*Activity not significantly above background

n.d. Not determined

The water and ethanol extracts from the roots of these plants were electrophoresed at pH 5.3. The activity patterns are shown in Figure IV-18. All patterns showed that most of the activity in the extracts appeared as compound B, with minor amounts only of compound A.

Partial analysis of the roots of these plants from two types of soil are given in Appendix 3. This shows much lower values of the trace elements chromium, nickel and cobalt in the roots from Manawatu soil than in the roots of the plants from serpentine soil. However, the large ash content (17%) of the roots from serpentine soil is probably indicative of soil contamination.

Fig. IV - 18. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis. Extracts of roots of soil-grown manuka plants fed $^{51}CrO_4^{2-}$.

(a) Plant from serpentine soil : ethanol extract.

(b) Plant from serpentine soil : water extract.

(c) Plant from Manawatu soil : ethanol extract.

(d) Plant from Manawatu soil : water extract.

(e), (f) Standard $^{51}CrO_4^{2-}$.

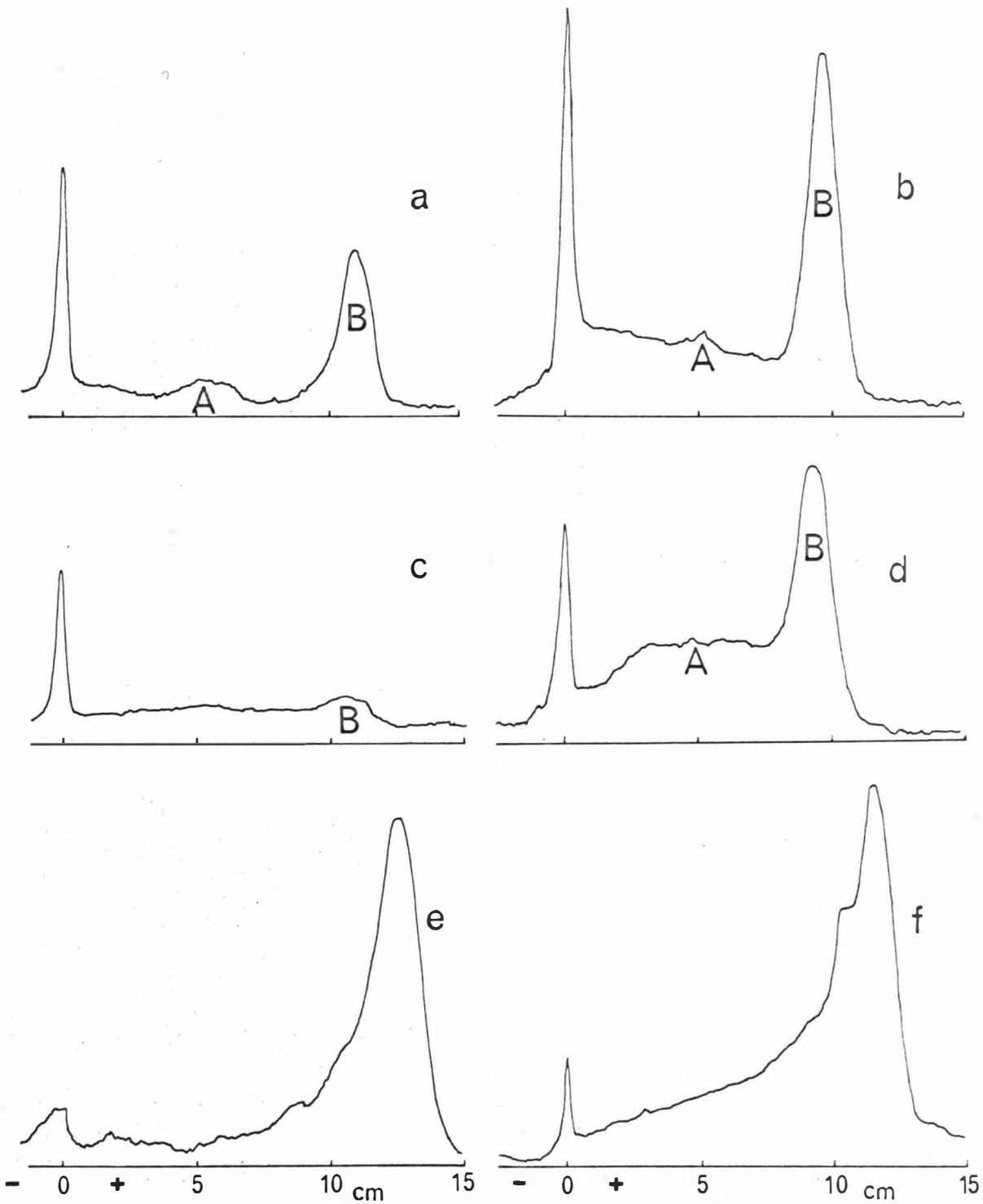

Figure IV - 18.

5. FURTHER EXPER S WITH MANUKA. TOSPERMUM SCOPARIUM

(i) Introduction

Earlier work, analytical (Part III), and the ^{51}Cr studies of the last section sidered three common indigenous species from the serpentine area: odora, C. vauvilliersii and manuka (L. scoparium). These results showed that manuka was able to accumulate and translocate chromium to the greatest degree. Only for manuka were any seeds available, and Mr D.A. Grant (pers. comm.) had shown that these seeds could be germinated under laboratory conditions.

Thus manuka was the most suitable species for further study. Manuka seeds unfortunately were not available from the serpentine area, but seed capsules were collected from mature trees near the Tiritea River to the west of Massey University. These were air dried and the small seeds obtained.

The previous work on clover, had shown few major differences between those plants which had been fed ^{51}Cr as chromic chloride, and those receiving sodium chromate. Thus it was decided that use of both these forms for further experiments was not justified. Almost all the experiments with manuka described in this section were carried out using only $Na_2{}^{51}CrO_4$.

(ii) Culture and Feeding

Manuka seedlings were cultured under the same conditions as for clover. Seeds were sown on glass beads dampened with distilled water. After 14 days, some nutrient solution was added, and when 28 days old, seedlings were transferred to pots containing aerated full-strength nutrient solution.

For feeding with radioisotope, the roots of the plants were washed with distilled water and transferred to a solution of ^{51}Cr in distilled water.

Partial analyses of the ash of manuka seedlings grown in nutrient solution without any added chromium, nickel or cobalt, are given in Appendix 3.

(iii) Uptake of Chromium-51

Twenty plants (74 days old) were fed a sodium chromate solution with a chromium content of 0.07 ppm. There were four dishes each containing five plants, and each with 200 μCi of ^{51}Cr. After 24 hours in this solution the plants were washed with distilled water, and were harvested and freeze-dried.

Two plants were pressed and the radioautograph of one is shown in Figure IV-19. As most of the radioactivity was in the roots, they were exposed only 1/150th of the time required to expose the shoots. However, ^{51}Cr is found in the shoots, and as with clover, it appeared to be concentrated at the edges of the leaves.

The remaining eighteen plants were assayed for radioactivity on the automatic counter, and activities, specific activities and shoot:root ratios calculated. These results are presented in Table IV-9. They again emphasise that the roots contained the higher activity. Although there were differences of total uptake between the individual plants, specific activity values varied to a lesser extent.

In a later experiment, one six-month-old manuka (as in Figure IV-28) was fed 7 mCi ^{51}Cr as chromic chloride (0.1 ppm total Cr)

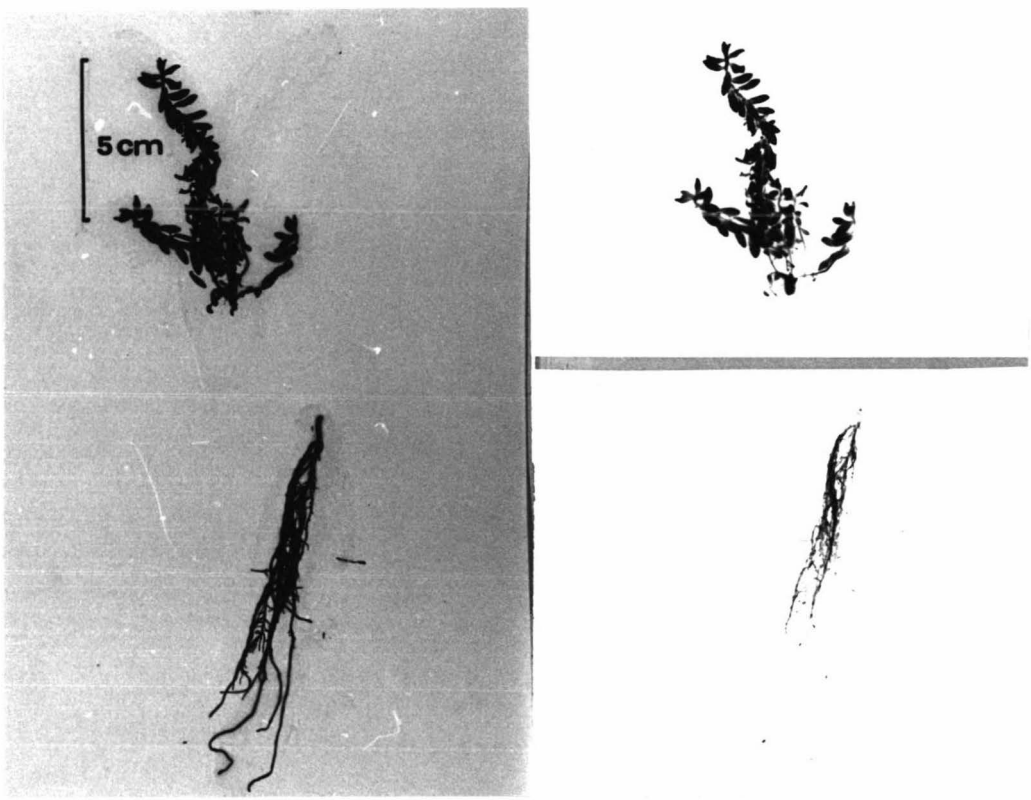

Fig. IV - 19. Leptospermum scoparium (manuka) from nutrient culture solution.
Left : photograph. Right : radioautograph.

Table IV - 9

CHROMIUM-51 RADIOACTIVITY IN MANUKA FROM NUTRIENT SOLUTION

(Activity in freeze-dried material; 18 samples)

		Shoots	Roots	Shoot:Root ratio
Total Activity (c/m)	Mean	20.5×10^{3}	274×10^{3}	67.8×10^{-3}
	Std. Dev.	8.5×10^{3}	91×10^{3}	23.6×10^{-3}
Specific Activity (c/m/mg)	Mean	267	17.3×10^{3}	14.6×10^{-3}
	Std. Dev.	161	7.6×10^{3}	4.4×10^{-3}

for 48 hours. Specific activities of leaves, stems and roots were determined as follows: leaf 10.3 c/m/mg, stem 4.5 c/m/mg, root 10400 c/m/mg. However the different conditions of age and feeding prevent direct comparison with plants fed $^{51}CrO_4^{2-}$.

(iv) Chemical Fractionation

The roots and shoots of plants from the above experiment were powdered, and were then subjected to the same chemical fractionation method as for clovers. The successive extraction system modified from that of Bowen et al (1962) as in Figure IV-5, gave the results shown in Table IV-10. Recoveries of 96.8% of the shoot activity and 89.3% of the initial root activity were made.

When these results are compared with those for the clover (Table IV-2), several major differences are observed. Of note are the large proportions of the radioactivity in the perchloric acid (D) and caustic soda (E) fractions from the manuka shoots. From the manuka roots however, a very high proportion (32%) was soluble in boiling 80% ethanol. Furthermore, nearly 60% of the ^{51}Cr was readily soluble (Fractions A, B and C), indicating that the ^{51}Cr was not tightly bound in manuka roots, certainly less so than in the shoots. This root ethanol fraction, which contained the largest amount of readily soluble ^{51}Cr, was further studied to determine the chromium compounds present, and is considered in the next section.

Preparations of RNA and of protein as for the clovers, were carried out on manuka shoots and roots. The results in Table IV-11 show that, as with clovers a negligible proportion of the radioactivity was associated with these fractions.

Table IV - 10

CHEMICAL FRACTIONATION OF CHROMIUM-51 IN MANUKA FROM NUTRIENT SOLUTION

(Percentage of counts recovered)

Fraction	A	A_1	B	C	C_1	D	D_1	E	F
Solvent	Ethanol		Water	HCl		$HClO_4$		NaOH	
Shoots	9.1	0.4*	5.8	3.0	0.2*	38.5	0.7	38.8	3.5
Roots	32.5	n.d.	14.3	12.8	0.6	16.5	0.3	15.3	7.7

* Activity not significantly above background

n.d. Not determined

Table IV - 11

CHROMIUM-51 RADIOACTIVITY IN PROTEIN AND RNA FROM MANUKA

(Percentage of total activity)

Part of plant	RNA	Protein
Shoots	0.12	<0.01
Roots	0.10	0.03

(v) The Chemical Form of Chromium-51 in Manuka

The boiling 80% ethanol extract of chromate-fed manuka roots was concentrated under vacuum at < 40°C. Aliquots of this concentrate were subjected to high voltage electrophoresis at pH 5.3 as described earlier. A densitometer scan of the radioautograph of the electrophoretogram is shown in Figure IV-20. Standard samples of $Na_2{}^{51}CrO_4$ were electrophoresed simultaneously and the pattern produced is also shown in the figure.

It can be seen that most of the radioactivity in the extract was anionic at pH 5.3, though some was immobile, but the ^{51}Cr was not present as $^{51}CrO_4^{2-}$. Most (44%) of the activity was confined to a very fast compound (C), with lesser amounts in other, confined areas (A and B) which were slower than ^{51}Cr-chromate. This pattern was consistent for the ethanol extract of all nutrient grown manuka plants

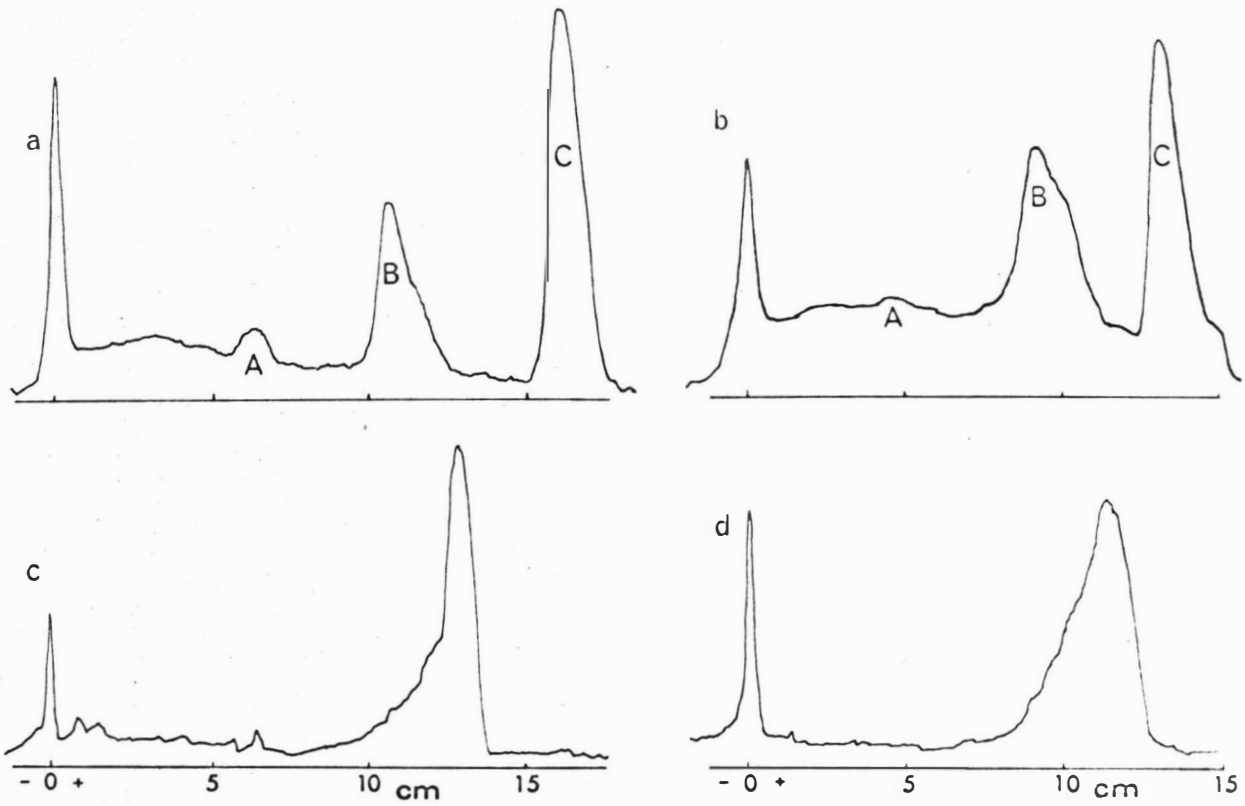

Fig. IV - 20. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis. Extracts of roots of nutrient-grown manuka fed with $^{51}CrO_4^{2-}$.

(a) Ethanol extract.

(b) Water extract.

(c), (d) Standard $^{51}CrO_4^{2-}$.

which had been fed with ^{51}Cr-sodium chromate. However the boiling water extract of the same manuka roots contained relatively less (26%) of the most mobile compound (C) than did the ethanol extract, although there were similar amounts of compound B (Figure IV-20).

To identify these compounds by an instrumental method of analysis or by spot tests, was considered very difficult because of the extremely low concentration of chromium present in the plant.

As a first attempt, various mixtures of ^{51}Cr and organic acids were made, samples of these mixtures were electrophoresed at pH 5.3, and the patterns compared with those of the ethanol extract. Solutions of chromic chloride, chromic sulphate, sodium chromate and sodium dichromate, each spiked with $Na_2{}^{51}CrO_4$ were allowed to equilibrate with equimolar solutions (0.1 M) of succinic acid, oxalic acid, disodium ethylenediaminetetracetic acid (EDTA), citric acid, l-malic acid and tartaric acid. Of these, only mixtures of oxalic acid or citric acid with chromium chloride solution showed any anionic activity at pH 5.3, as in Figure IV-21. Similar mixtures of chromic chloride with L-aspartic acid, L-glutamic acid, L-cysteic acid and malonic acid were also prepared and electrophoresed at pH 5.3. The mixture with malonic acid was the only one to show evidence of a chromium-containing anion (also in Figure IV-21). However, only with oxalic acid was there any ^{51}Cr radioactivity in a peak faster than ^{51}Cr-chromate.

From a comparison of the electrophoretic mobilities, it appears that compound C could be a complex of chromium and oxalic acid, and that compound A may be a complex of chromium and either citric acid or malonic acid.

Fig. IV - 21. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis.

Mixtures of $^{51}CrCl_3$ with organic acids.

(a) Citric acid.

(b) Malonic acid.

(c) Oxalic acid.

(d) Standard $^{51}CrO_4^{2-}$.

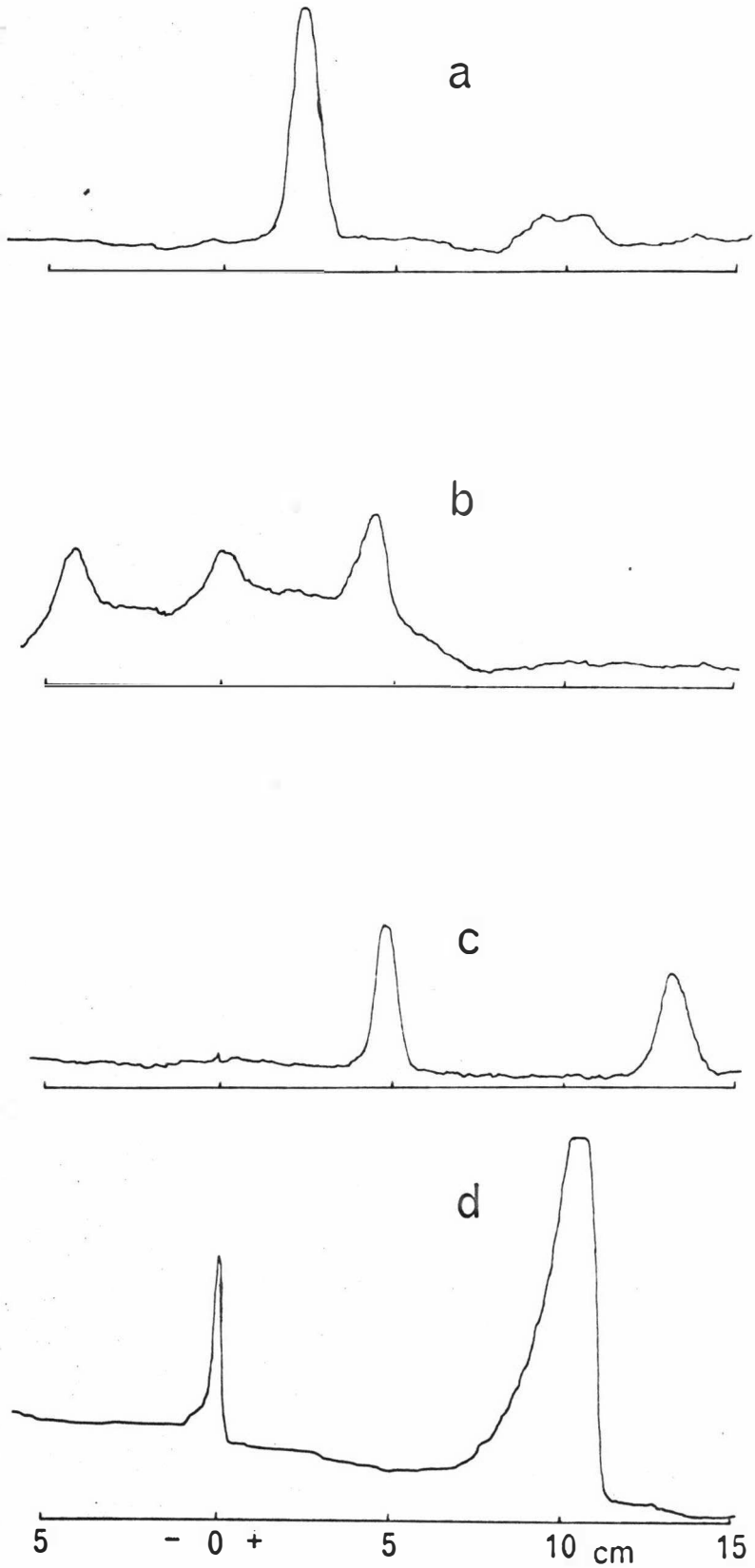

Figure IV - 21.

No electrophoretically-mobile ^{51}Cr compounds were detected in the ethanol extract of the roots of a $^{51}Cr^{3+}$-fed manuka.

(vi) The Oxalic Acid Complex

Oxalic acid is well known as a chelating group for chromium. The complex was prepared on a microscale, using ^{51}Cr-chromate, by the standard method (Palmer, 1965, p. 386). Initially oxalic acid, in concentrated solution at 70°, reduces chromate:

$$2CrO_4^{2-} + 3(COOH)_2 + 10H^+ \longrightarrow 2Cr^{3+} + 6CO_2 + 8H_2O$$

In the presence of excess oxalic acid, the chromium (III) complexes with oxalate ions rather than water

$$Cr^{3+} + 3C_2O_4^{2-} \longrightarrow [Cr(C_2O_4)_3]^{3-}$$ trioxalatochromate (III) ion

Chromium (III) is always octahedrally coordinated, and in this compound, the three bidentate oxalate groups occupy the six positions. Electrophoresis of this preparation, which could not be purified by recrystallisation because of the small amount of product (< 5 mg) was made in pH 5.3 buffer, and a similar pattern to that in Figure IV-21 obtained.

This has two peaks of radioactivity, the fast one being the trioxalatochromate (III) ion, with three negative charges. The other compound present will be the diaquodioxalatochromate (III) ion $[Cr(C_2O_4)_2(H_2O)_2]^-$ with only one negative charge, where one oxalate group has been replaced by two water molecules. The trioxalate complex could be purified by a semi-preparation scale electrophoretic separation at pH 5.3. Localisation of the fast compound by radioautography was followed by elution of the compound with ethanol in the presence of ammonia. Without the ammonia, the free acid of

trioxalochromate (III) tended to decompose. Further comparisons of this compound were made with compound C isolated from manuka roots.

Both electrophoresis at pH 2.0 and chromatography in butanol: acetic acid:water showed that the pattern for the synthetic trioxalatochromate complex matched that of the major ^{51}Cr component of the ethanol extract of manuka roots.

Gel filtration was also attempted. A small column was prepared using 1 g of Sephadex G-10 (Pharmacia, Sweden). Dye was added to samples to locate the solvent front. One drop fractions were collected on planchets, and their radioactivity was determined using a Beckman Lowbeta counter, with a background of 1 cpm. A graph of drop number against activity for a sample of manuka root ethanol extract is shown in Figure IV-22. Also shown is the result from a similar run using a sample of standard chromium oxalate mixture. Very poor separation of any of the radioactivity from the solvent front was obtained for either the extract or the oxalate complex. The metal complex due to its small size (MW = 315) should have been retarded by the gel, but the retardation was not very significant. However, Spitzy et al (1961) have reported that Sephadex gel repels anions which would therefore be carried with the solvent front.

(vii) Experiment with Chromium-51 and Carbon-14

The above experiments indicated that compound C was the trioxalatochromate (III) ion but sufficient material could not be obtained to decompose the complex and identify the chelating group by any known spot tests.

As an alternative to isolating measurable amounts of this compound, a feeding experiment was carried out using ^{14}C as well as ^{51}Cr, to try and label the chelating group with ^{14}C.

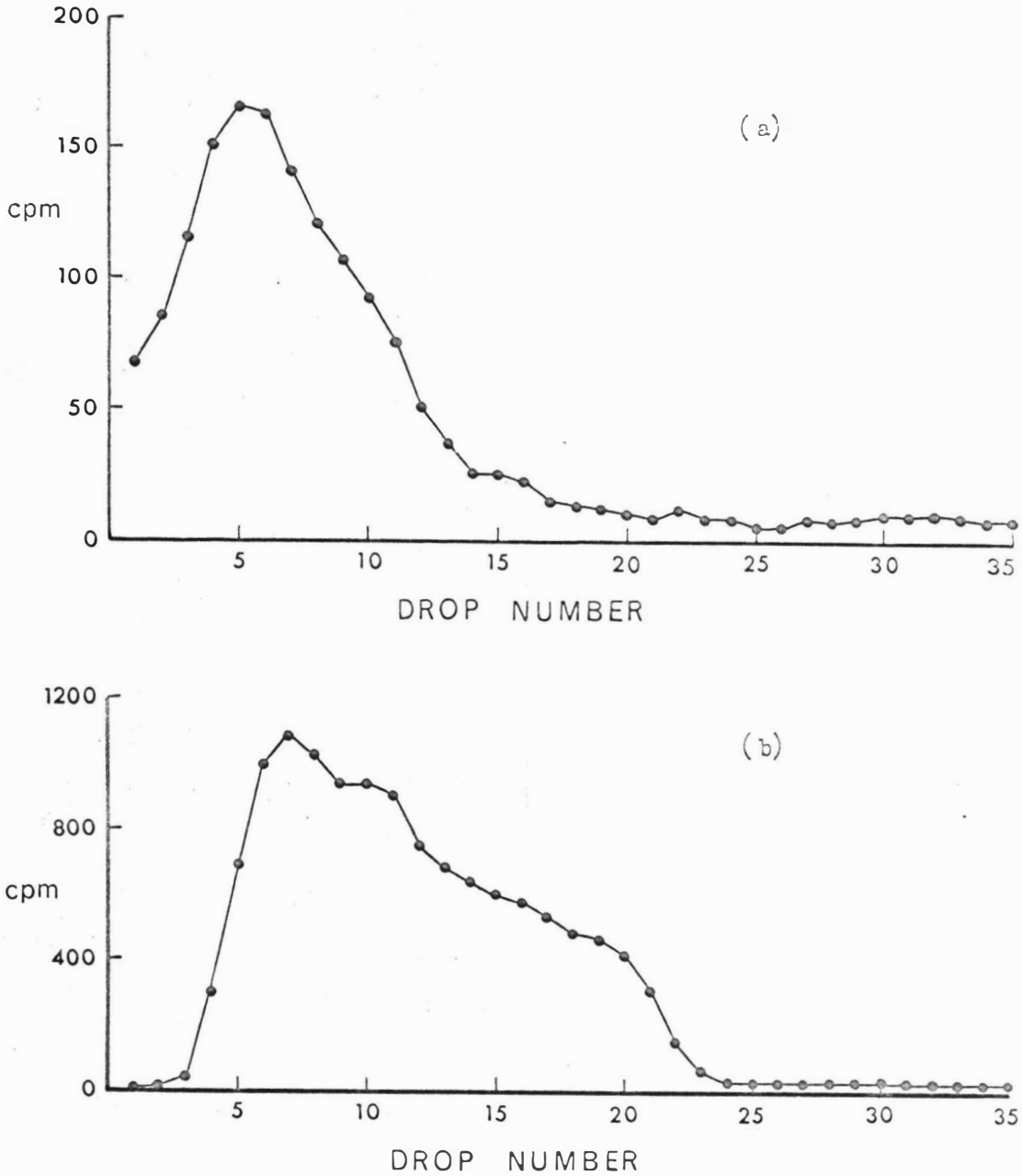

Fig. IV - 22. ^{51}Cr radioactivity of samples from a C1C Sephadex column.

(a) Ethanol extract of roots of a nutrient-grown manuka which had been fed with $^{51}CrO_4^{2-}$.

(b) Standard ^{51}Cr-trioxalatochromate(III).

(a) Uptake of Chromium-51 and Carbon-14

Five manuka plants (57 days old), grown from seed in nutrient solution were transferred, after washing, into a solution of 0.005M $Ca(NO_3)_2$ containing 3.5 mCi of ^{51}Cr as sodium chromate (0.1 ppm total chromium). These plants were then arranged in a sealed dessicator in an atmosphere containing 5 mCi of $^{14}CO_2$ released by the action of orthophosphoric acid on $Ba^{14}CO_3$. These plants remained 3 days in the $^{14}CO_2$ atmosphere and ^{51}Cr solution until the $^{14}CO_2$ was removed, and after a further two days, the roots were washed and the plants harvested and freeze-dried. No evidence of radiation damage was observed.

(b) Detection of Carbon-14 and Chromium-51

As ^{14}C is a β-emitter, the radiation is readily absorbed by any solid material, whereas the γ-radiation emitted by ^{51}Cr will penetrate a greater distance. Radioautographs of electrophoresis patterns containing ^{14}C and ^{51}Cr were made by placing two sheets of film on the paper. The sheet in contact recorded both ^{14}C and ^{51}Cr radioactivity but the second sheet would only record the ^{51}Cr γ-radiation which passed through the contact sheet. For quantitative measurement, a geiger counting tube was used with the Philips measuring equipment to measure the radioactivity of the sample with and without a piece of card between the sample and detector. Comparison of the measurements from standard samples of ^{14}C, ^{51}Cr, and a mixture of ^{14}C and ^{51}Cr, showed that none of the ^{14}C activity was measured when the card was in place, whereas 44.5% of the ^{51}Cr activity was recorded.

(c) Extracts

Samples of these radioactive plants, which were separated into leaves, stems and roots, were extracted with boiling 80% ethanol. The extracts were concentrated and after removal of the ether-soluble

components from the leaf extract, were electrophoresed at pH 5.3. The residues from the ethanol extracts were then extraced with boiling water. These extracts were freeze-dried and also electrophoresed at pH 5.3.

The water and ethanol electrophoretograms were all radioautographed to give two patterns, one for only ^{51}Cr and the other for the sum of ^{51}Cr and ^{14}C activity. A further ^{14}C radioactivity pattern was obtained by a contact radioautograph three months later when only 10% of the ^{51}Cr would still be present. The ^{51}Cr and the ^{14}C patterns are shown in Figures IV-23 and IV-24.

The root ethanol extract, and the leaf water extract both showed most (42%) of the ^{51}Cr radioactivity as compound C, and about 25% of the activity as compound B. However, the leaf and stem ethanol extracts, both of which showed compound A as a broad or double peak due to overloading of the electrophoretogram, did not have compound C. Also, as in some of the other electrophoretograms (Figures IV-7a, IV-18a, IV-20a and IV-20b), the peak labelled compound B showed a distinct shoulder on the leading edge indicating the presence of another component, possibly chromate, in the extract.

The ^{14}C radioactivity in all cases was spread over a large number of peaks of the electrophoresis pattern, very few corresponding to any of the ^{51}Cr peaks.

The ethanol extract of the manuka root material was then electrophoresed as a band at pH 5.3, compound C was localised by radioautography and eluted from the paper with water.

This solution was concentrated and an aliquot counted on the geiger tube for ^{14}C and ^{51}Cr. Determination of the activity of the ethanol extract gave a $^{14}C/^{51}Cr$ count ratio of 60.9 but the purified compound C contained very little of the ^{14}C, with a $^{14}C/^{51}Cr$ count ratio of 0.45 (corrected for decay).

Fig. IV - 23. ^{51}Cr and ^{14}C radioactivity patterns after pH 5.3 electrophoresis.

Ethanol extracts of nutrient-grown manuka.

(a) Leaf ^{14}C.

(b) Leaf ^{51}Cr.

(c) Stem ^{14}C.

(d) Stem ^{51}Cr.

(e) Root ^{14}C.

(f) Root ^{51}Cr.

(g) Standard $^{51}CrO_4^{2-}$.

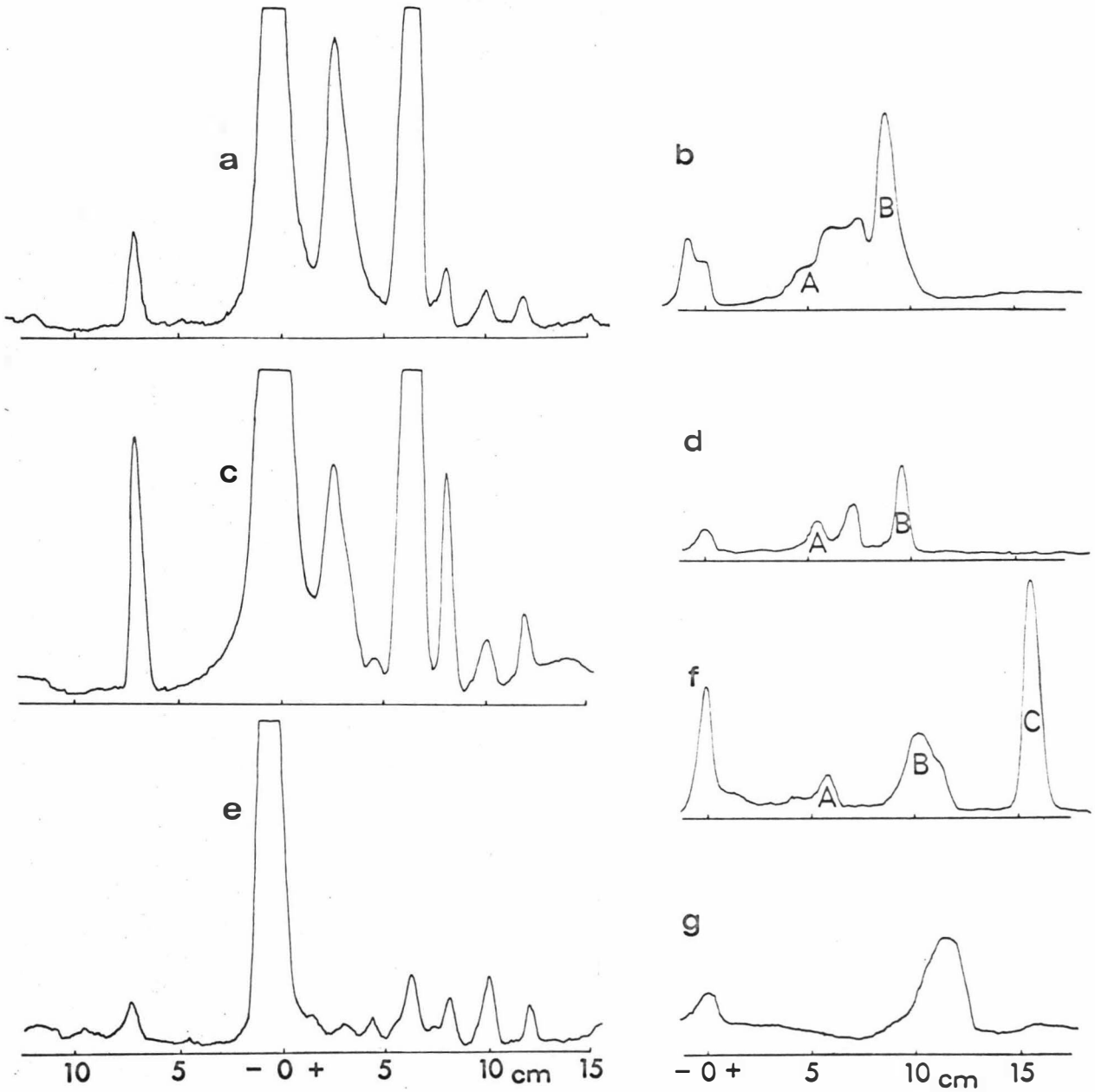

Figure IV - 23.

Fig. IV - 24. ^{51}Cr and ^{14}C radioactivity patterns after pH 5.3 electrophoresis.

Water extracts of nutrient-grown manuka.

(a) Leaf ^{14}C. (b) Leaf ^{51}Cr.

(c) Stem ^{14}C. (d) Stem ^{51}Cr.

(e) Root ^{14}C. (f) Root ^{51}Cr.

(g) Standard $^{51}CrO_4^{2-}$.

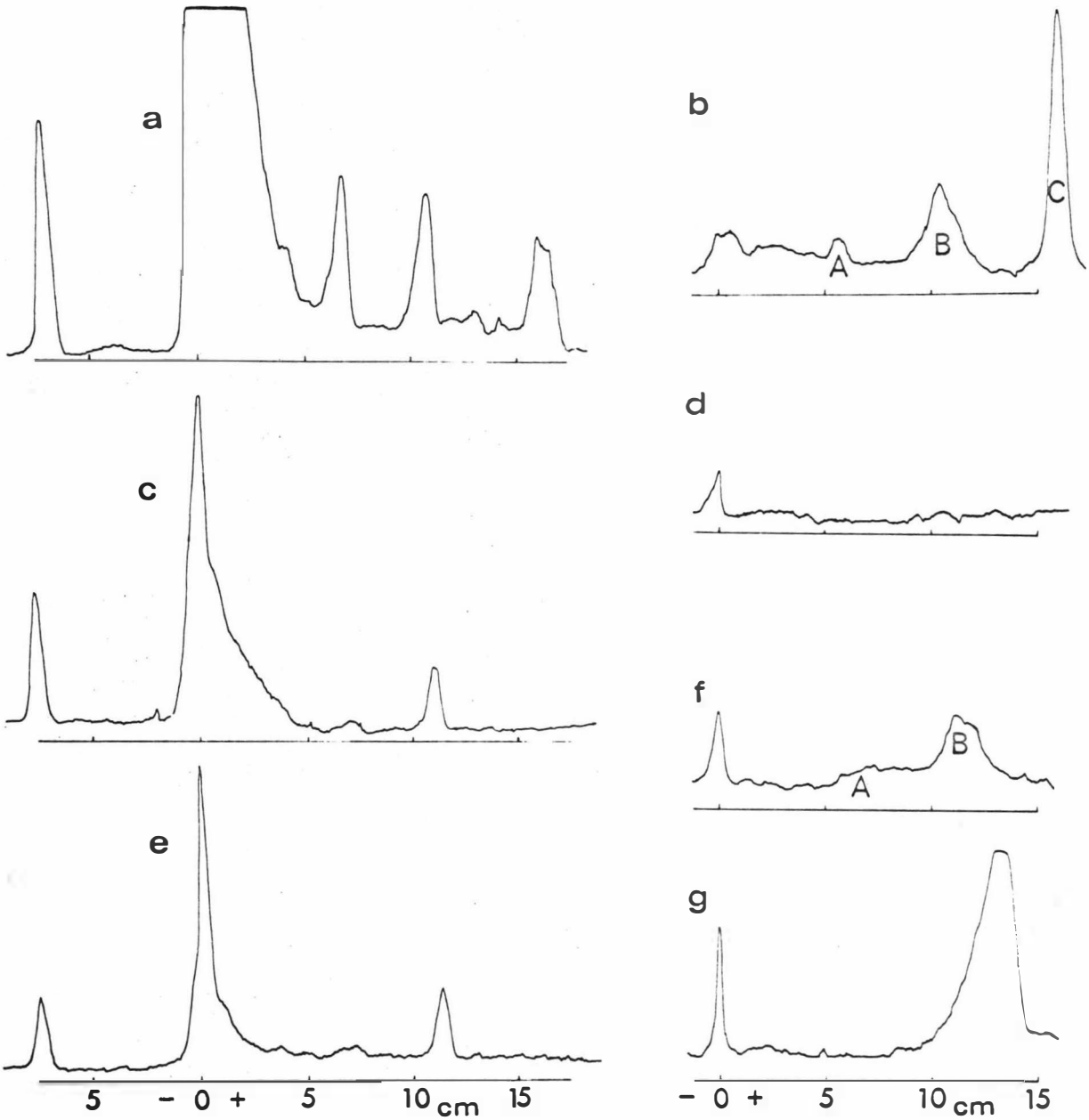

Figure IV - 24.

A preparation was also made of potassium tri-^{14}C-oxalate-^{51}Cr-chromate (III) using 0.1 mCi of ^{14}C-oxalic acid (7.5 mg) and 1 mCi of sodium ^{51}Cr-chromate (3 mg) by the same method as before (Palmer, 1965). However, on pH 5.3 electrophoresis, this preparation showed the presence of unreacted ^{51}Cr-chromate, as well as some of the dioxalate complex but was still able to be used as a standard for most purposes. Later this was purified as with earlier preparations of the chromium oxalate complex, and an ammonium tri-^{14}C-oxalate-^{51}Cr-chromate (III) solution was used as a standard.

Hydrolysis of compound C with 6N hydrochloric acid at 70°C overnight and subsequent concentration and electrophoresis at pH 2.0 showed (Figure IV-25) that the ^{51}Cr was no longer all in one major, fast anion, but as a number of smaller peaks. Similar hydrolysis of an aliquot of the purified trioxalate chromate, and electrophoresis, again showed destruction of the original compound, and the production of a number of weaker ^{51}Cr peaks. By comparison with the hydrolysate from compound C, at least three and possibly four peaks appeared to match. Unfortunately, most of the ^{14}C from the compounds was volatile although some may have still been present as the only anion in the compound C hydrolysate.

Thus this experiment was not completely successful in isolating and identifying a ^{14}C-labelled chelate, but the dissociation pattern of ^{51}Cr label from compound C is similar to that of the trioxalato-chromate (III) ion.

Fig. IV - 25. Patterns of radioactivity (^{14}C+^{51}Cr) after pH 2.0 electrophoresis.

A) Standard $\left[^{51}Cr(^{14}C_2O_4)_3\right]^{3-}$.

B) Compound C (separated by preparation-scale electrophoresis).

C) Standard $^{51}CrO_4^{2-}$.

D) HCl-hydrolysed $\left[^{51}Cr(^{14}C_2O_4)_3\right]^{3-}$.

E) HCl-hydrolysed compound C.

F) Standard ^{14}C-oxalic acid.

G) Standard $^{51}CrO_4^{2-}$.

H) Standard $^{51}Cr^{3+}$.

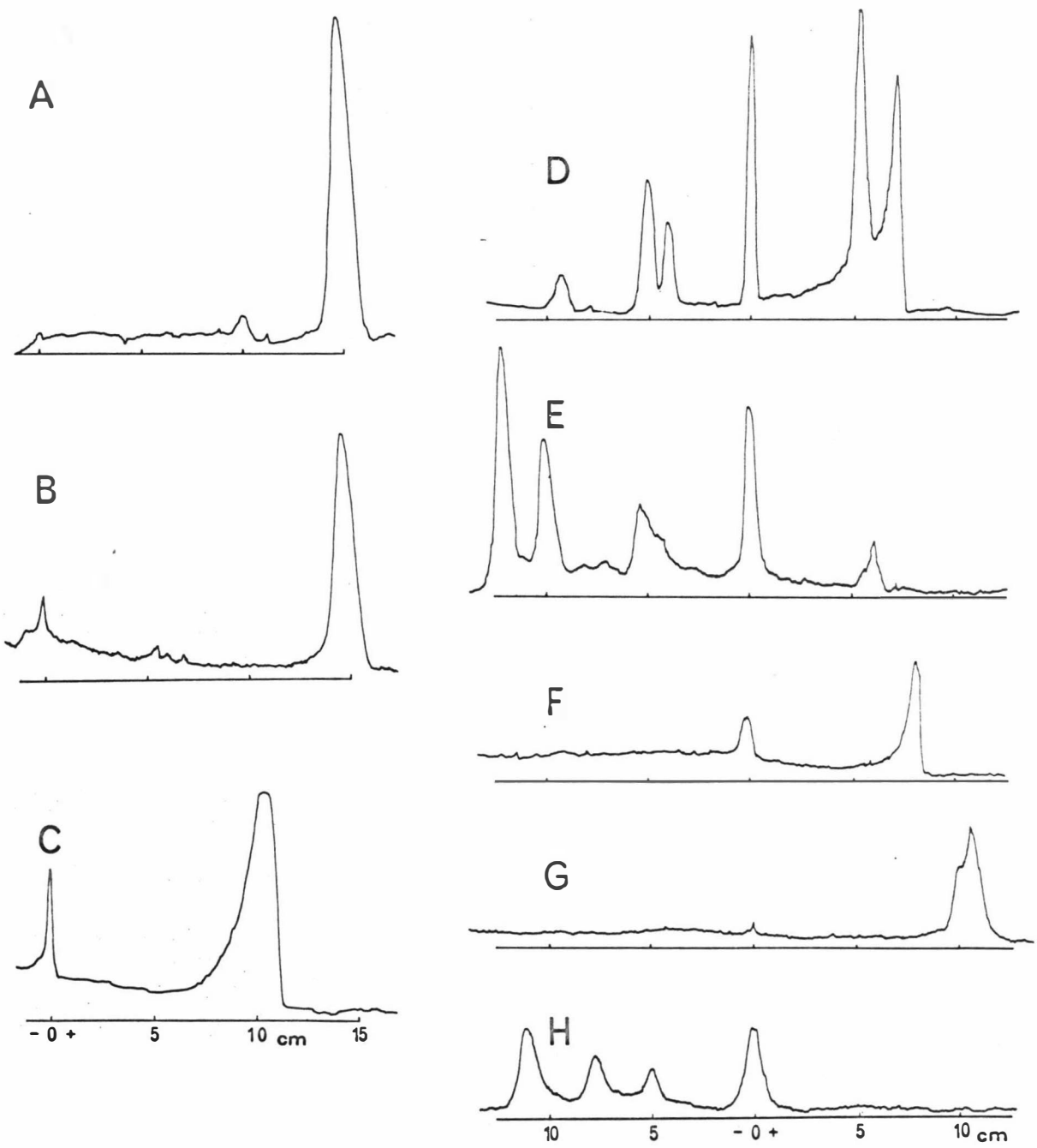

Figure IV - 25.

6. CHROMIUM-51 IN XYLEM SAP

The previous experiments have given information on the chemical forms of chromium in the readily soluble fractions of both roots and aerial tissues of various plants. The experiments with manuka showed that the major chromium component of the ethanol extract of roots was the trioxalatochromate complex, which does not appear in the leaf and stem ethanol extracts. It is therefore of great interest to know the chemical state of chromium when it is being transported from the roots to the leaves. A study of the chromium composition of the xylem sap of manuka plants was therefore undertaken.

(i) Experimental

An apparatus for the removal of xylem sap was built at Plant Chemistry Division, D.S.I.R., Palmerston North (Figure IV-26) and is similar to that described by Scholander *et al* (1964). The method of extraction of the xylem sap was rapid. A leafy twig was taken, the bark and phloem were peeled back from the cut end with a razor blade, and the stem placed in the lid of the pressure bomb (see figure). This cut stem was sealed by the rubber compression gland, with the cut end free. The lid was fitted to the bomb and nitrogen gas was pumped in until sap flowed out the cut end. The sap was collected by fitting a polythene tube to the end of the stem and leading it to a test-tube. In use, xylem sap started to flow at 20-30 atmospheres gas pressure, and a quantity was collected at a pressure of 100 atmospheres.

The sap collected from manuka was shown to be xylem sap by a study of its amino acid composition by chromatography, and electrophoresis. This showed the dominance of glutamine, with only minor

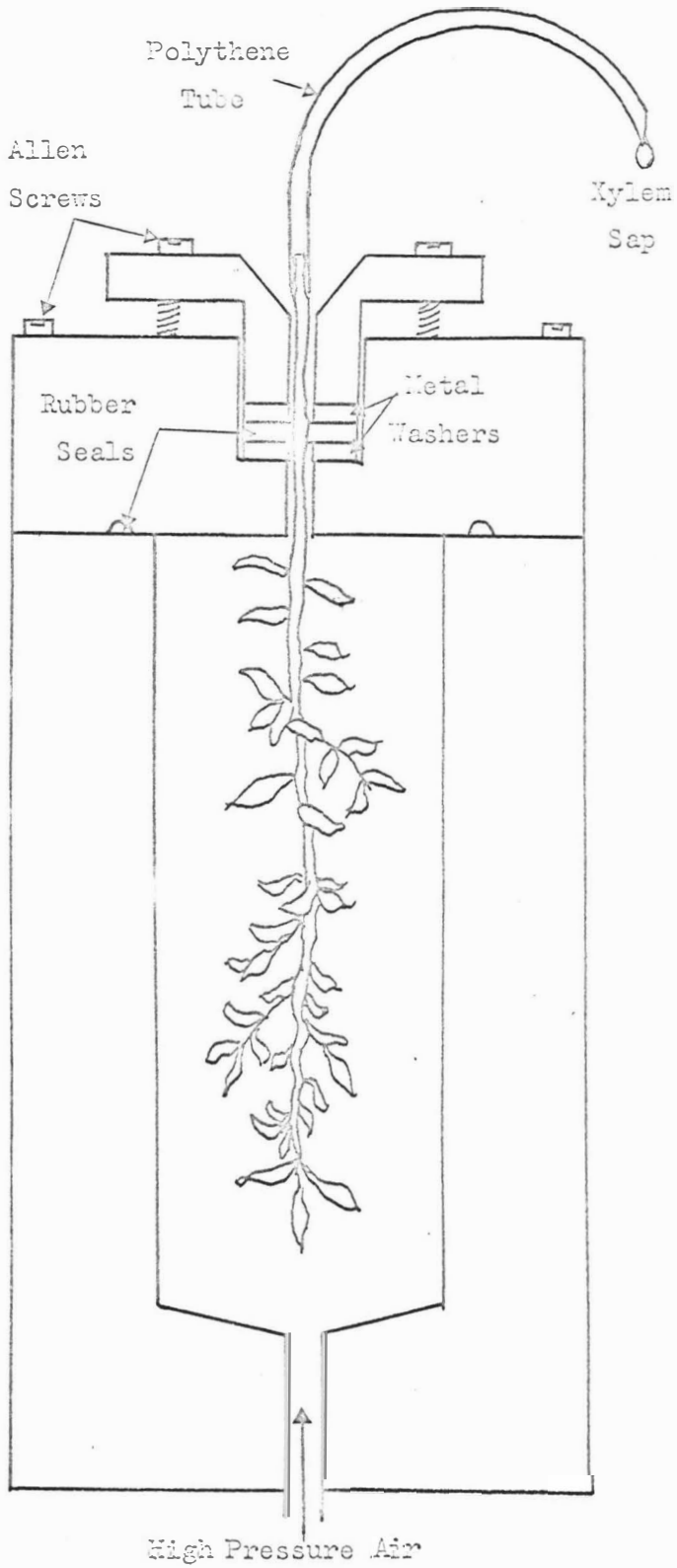

Fig. IV - 26. Apparatus for the removal of xylem sap.

amounts of other amino acids such as glutamic acid, alanine, valine, serine, threonine, arginine, aspartic acid, asparagine and Y-amino-butyric acid (P.J. Peterson, pers. comm.).

(ii) Manuka Plants from Serpentine Soil

Four manuka seedlings, which had been kept in a glasshouse for two years, two plants in serpentine soil, and two in Manawatu silt-loam, were removed from their pots. The soil was washed from the roots, and the plants were placed in a 0.005M $Ca(NO_3)_2$ solution. After two hours washing in this solution the plants were transferred to a fresh solution containing 0.1 ppm chromium as $Na_2{}^{51}CrO_4$ (4 mCi ^{51}Cr).

After 48 hours in this aerated solution in the growth cabinet, the stems were severed from the roots and each shoot was placed in the bomb as described in the previous section. Xylem sap was also pumped out of the roots by the same method.

Each of these samples was concentrated and the concentrates were electrophoresed at pH 5.3. Figure IV-27 shows some of the patterns of ^{51}Cr radioactivity; all others were similar. All show the undoubted presence of the ^{51}Cr-chromate ion. There is no apparent difference between those plants which had been acclimatised to Manawatu soil, and those which had not.

(iii) Manuka Plants from Nutrient Solution

(a) Chromate-fed

A manuka plant, which had been grown from seed and cultured in nutrient solution in the growth cabinet for six months, was transferred (after washing) into a 0.005M $Ca(NO_3)_2$ solution containing

Fig. IV - 27. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis.

Xylem sap from soil-grown manuka fed with $^{51}CrO_4^{2-}$.

A) Plant from Manawatu soil : sap from shoot.

B) Plant from Manawatu soil : sap from roots.

C) Plant from serpentine soil : sap from shoot.

D) Plant from serpentine soil : sap from roots.

E) Standard $^{51}CrO_4^{2-}$.

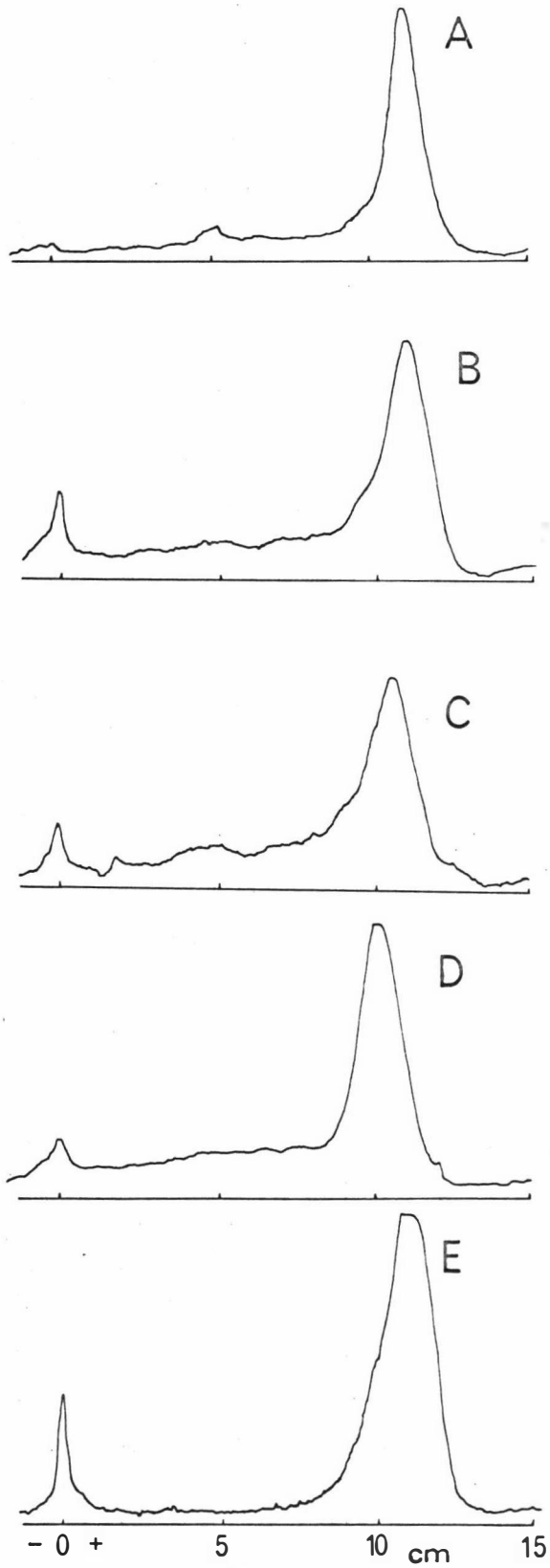

Figure IV - 27.

0.2 ppm chromium as sodium ^{51}Cr-chromate (10 mCi ^{51}Cr). A photograph of this plant (Figure IV-28) shows that it is much larger than the plants used in the last section, which were similar in size to those shown in Figures IV-12 to IV-14, and were less than 25 cm tall.

Unfortunately, during photography, this plant may have become dehydrated. After 32 hours in ^{51}Cr solution, it was removed and sap pressed from shoots. Only 100 µl of solution was obtained from 8.0 g (fresh weight) of shoot material, much less than other similarly grown manukas.

This extracted liquid was concentrated and electrophoresed at pH 5.3. Figure IV-29 shows that, as with the soil grown plants (Figure IV-27) the ^{51}Cr is present as the chromate ion.

(b) Chromium(III)-fed

A similar plant, of the same age, was fed 10 mCi ^{51}Cr as chromic chloride, under similar conditions to previously (0.2 ppm chromium as $CrCl_3$ in 0.005M $Ca(NO_3)_2$). After 48 hours in ^{51}Cr solution, 1.6 mls of sap was collected from 12.2 g (fresh weight) of shoot tissue at a maximum pressure of 50 atmospheres. This solution was concentrated and an aliquot electrophoresed at pH 5.3. The radioactivity pattern (Figure IV-29) however, shows no evidence for any ^{51}Cr cations, but a broad anionic peak of activity. This anionic form gave a very poor pattern on electrophoresis and did not appear to match the pattern produced by chromate.

(c) Oxalic Acid Composition

In view of the fact that chromium occurs as a complex with oxalic acid in the roots of nutrient-grown manuka plants, it was of

Fig. IV - 28. Leptospermum scoparium (manuka) growing in nutrient solution. (Scale in inches)

Fig. IV - 29. Pattern of ^{51}Cr radioactivity after pH 5.3 electrophoresis.

Xylem sap from nutrient-grown manuka.

A) Sap of a plant supplied with $^{51}CrO_4^{2-}$.

B) Standard $^{51}CrO_4^{2-}$.

C) Sap of a plant supplied with $^{51}Cr^{3+}$.

D) Standard $^{51}Cr^{3+}$.

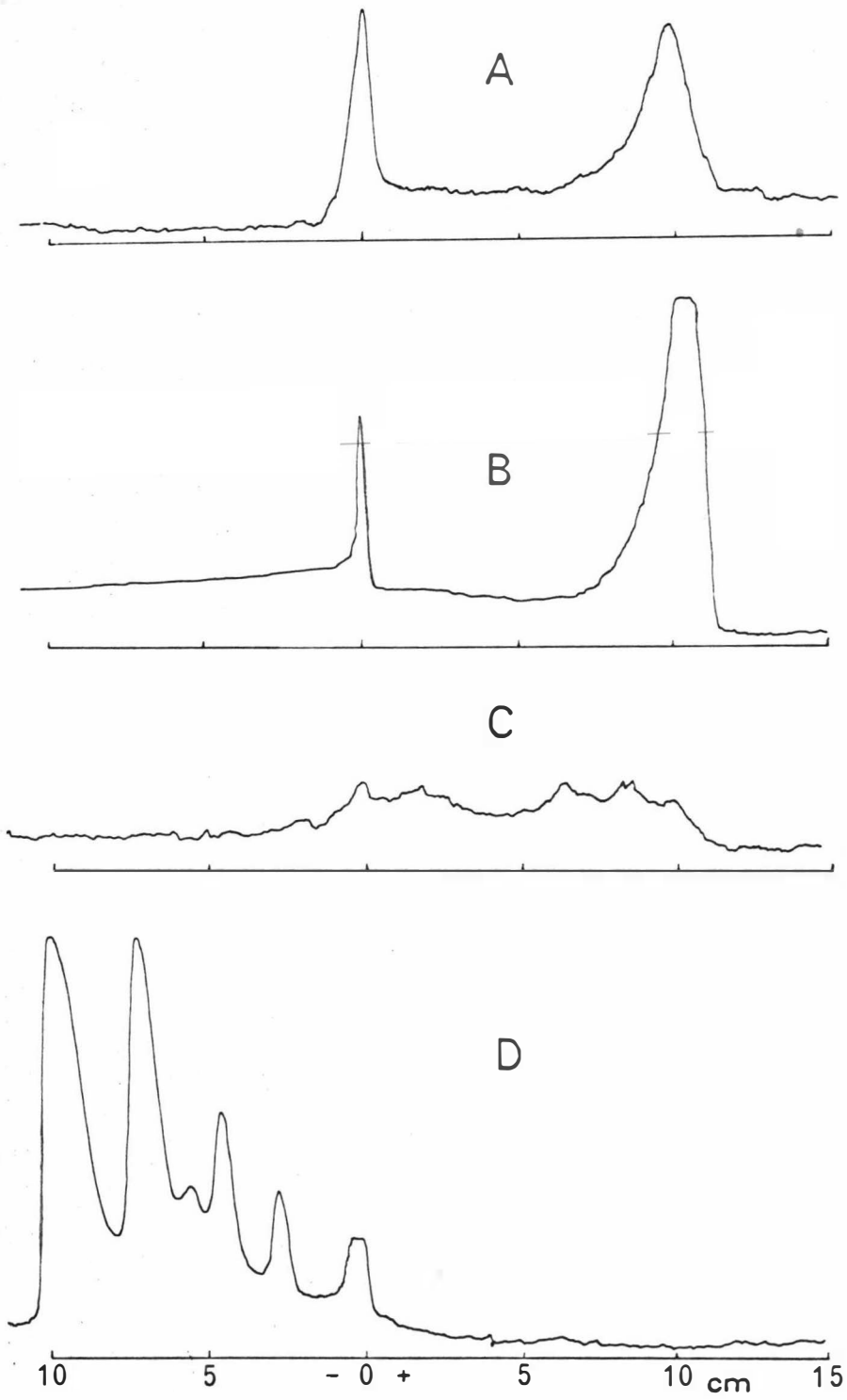

Figure IV - 29.

interest to see if oxalic acid was present in the sap which carried chromium as ^{51}Cr-chromate.

With plant extracts, oxalic acid determination is very difficult due to the presence of many other substances but xylem sap is relatively free of sources of interference. Experiments showed that oxalic acid, on electrophoresis at pH 2.0, is a very mobile anion and separates from other organic acids. Using aniline-xylose reagent (Nordmann and Nordmann, 1960), 10 µg of oxalic acid showed a pronounced dark spot and 2 µg could be detected.

An aliquot of xylem sap from a nutrient-grown manuka which had not been fed chromium, was electrophoresed at pH 2.0 along with standards. In this sample no oxalic acid could be detected. This corresponds to a concentration in the xylem sap of less than 10 µg/ml oxalic acid. Other organic acids were present at approximately ten times this concentration.

7. DISCUSSION

No matter whether chromium was supplied as Na_2CrO_4 or as $CrCl_3$, nor which species of plant was being considered, in all solution culture experiments, a very large accumulation of ^{51}Cr in roots was observed compared with that in the aerial parts of the plant. Very little of this root radioactivity was able to be translocated upward by clovers when they were removed from the ^{51}Cr solution and returned to normal nutrient culture.

These results indicate a similar effect to that observed by DeKock (1956) with mustard plants, which when supplied with chromium in solution culture increased the root concentration of chromium to 20,000 ppm while the leaf concentration increased to only 20 ppm. Control plants contained about 10 ppm in all parts of the plants, but the ones fed 2 ppm Cr in culture solution, either as $CrCl_3$ or Cr-EDTA, became chlorotic with decreased iron content and increased phosphorus levels.

This accumulative behaviour of chromium in roots suggests that precipitation or absorption of ^{51}Cr is occurring on or at the roots. This may be caused by precipitation by inorganic nutrient ions since, although the roots were washed before being placed in ^{51}Cr solution, there could be such ions as phosphate, ammonium, sulphate, calcium, and magnesium still in appreciable amounts in the root cells near the sites of entry of ^{51}Cr into the roots. However, the concentration of chromium in the feeding solution was never more than 0.1 ppm Cr(2 μM) and other ions are unlikely to be present in greater concentrations.

Therefore the chromates of copper and of other divalent heavy metals which are insoluble, with solubility products between 10^{-4}

and 10^{-10} (Sillen and Martell, 1964) are unlikely to precipitate since these elements are all at very low concentrations in plants. However, when ^{51}Cr is fed as Cr(III) or is reduced from Cr(VI) in the roots, precipitation as the phosphate could occur. Sillen and Martell (1964) quote solubility products of 10^{-23} and 10^{-17} for different forms of $CrPO_4$, but add that there is evidence for the formation of $[CrHPO_4]^+$ and $[Cr(PO_4)_2]^{3-}$ ions. This precipitation then, is possible, though doubtful as the PO_4^{3-} concentration will be very low, even in the nutrient solution where phosphorus is supplied as 2 mM KH_2PO_4.

As an alternative to chemical precipitation, ^{51}Cr may be absorbed onto cell walls or complexed with proteins or other carboxylic groups in the root cells. The extraction experiments, however, in all cases show no evidence of any substantial proportion of ^{51}Cr being bound to proteins, whether determined using the method of Bowen et al (1962) or a more specific separation. This therefore throws doubt on the statements of Bourque et al (1967) who considered they had evidence (undefined) that ^{51}Cr in excised wheat roots is predominantly bound to protein, although Pierce (1964) has recorded the binding capacity of human blood proteins to be more than 20 moles Cr(III) per mole of protein.

The most significant observation from the radioautographic studies, is that ^{51}Cr is able to be translocated from the nutrient solution, where it may be as Cr^{3+} or CrO_4^{2-}, to all parts of the plants. In detail, apart from the much higher concentrations of chromium in the roots compared with shoots, an interesting aspect is the relatively

greater amounts of radioactivity found at the edges of the leaves of clover, manuka and P. suteri, and, in clover, accumulations in senescent leaves.

Recently, Millikan et al (1968) have shown that accumulations of ^{65}Zn occur at the edges of leaves of Trifolium subterraneum under conditions of normal zinc and low phosphorus culture, but not at deficient zinc levels. Mitchell and Reith (1966) have shown that senescent leaves of cocksfoot, rye grass and of a mixed pasture in Scotland contained nearly 10 ppm of lead (on dry weight basis) in November compared with less than 1.0 ppm when the leaves were young in May. This may therefore be used by the plant as a mechanism for the removal of unwanted trace elements from the plant.

The experiments comparing the uptake of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ in clover plants showed that although CrO_4^{2-} was able to be accumulated by the clover roots faster than was Cr^{3+}, the ^{51}Cr fed as Cr^{3+} was translocated to the shoots faster than was ^{51}Cr fed as CrO_4^{2-}. Bourque et al (1967) found that excised wheat roots absorbed ^{51}Cr about fourteen times as much if supplied as CrO_4^{2-} than when supplied as Cr(III). From this, they considered that only CrO_4^{2-} was able to penetrate intact cells, and that the small amount of Cr(III) recovered was due to absorption at cut surfaces. This contradicts the experimental results in this thesis which show that ^{51}Cr can be assimilated and translocated whether as Cr^{3+} or CrO_4^{2-} in the feeding solution, although at different rates. The observed differences in uptake between the two forms may be explained, in part at least, by the differing charges and sizes of the $[Cr(H_2O)_6]^{3+}$ ion (4.0 Å diameter) and CrO_4^{2-} ion (3.2 Å diameter).

When fed ^{51}Cr-chromate, manuka plants were found to transport ^{51}Cr as chromate in the xylem sap. Thus it appears unlikely that all of the chromate is reduced in the roots, where this could explain the differing translocation rates of ^{51}Cr when fed as CrO_4^{2-} or Cr^{3+}. If chromate was reduced to Cr(III) on entry into the roots, this form would be readily chelated, either by water, or preferably by carboxylic compounds such as proteins (Pierce, 1964) or organic acids. Such chelation would be much slower with $[Cr(H_2O)_6]^{3+}$, as ligands of chromium (III) are characteristically inert and exchange of water molecules for other chelates is very slow (Cotton and Wilkinson, 1962, p. 542, 548). Differences in the effect of Cr^{3+} compared with CrO_4^{2-} have been shown by Hewitt (1963) with sugar beet receiving different amounts of molybdenum. CrO_4^{2-} depressed chlorophyll levels in plants fed with normal molybdenum concentrations compared with plants fed high molybdenum levels. However, with Cr^{3+} this chlorotic effect was reversed, those plants supplied with high molybdenum concentrations becoming more chlorotic.

Comparison of the proportion of ^{51}Cr in each of the fractions of the successive extraction procedure showed no major differences between clovers fed with $^{51}Cr^{3+}$ and those fed $^{51}CrO_4^{2-}$. However, there was invariably a greater proportion of ^{51}Cr soluble in water from the roots than there was from shoots, with generally 30-50% of ^{51}Cr extracted from clovers by ethanol and water.

The indigenous plants, H. odora and manuka, although grown under different conditions to the clover and of different ages, contained ^{51}Cr in generally similar proportions, in the various fractions, to those in clover. Chromium-51 from roots of H. odora was less soluble in the earlier fractions compared with other samples, probably because

of absorption on to soil particles. Stems of H. odora grown in serpentine soil contained an unusually high fraction of ^{51}Cr soluble in boiling water (38%), much more than the proportion from stems of soil-grown manuka plants. On the other hand, manuka shoots grown from solution culture contained 38% of ^{51}Cr in the perchloric acid fraction (D), a proportion greater than that from clovers fed similarly with $^{51}CrO_4^{2-}$. However, the roots from these manuka plants contained 32% of the chromium soluble in 80% ethanol, a greater proportion in this fraction than from any other of the samples studied.

These results may be compared with the study of 11 different isotopes in tomato leaves, by Bowen et al (1962) where they found that more than 60% of the radioactivity of the elements copper, magnesium, manganese, potassium, sodium, tungsten and zinc was removed in the hydrochloric acid fraction but only for cobalt and sodium was more than 20% extracted by ethanol. These authors did not separate a water soluble fraction, but components of this would be included in the hydrochloric acid extraction. In addition they found that for calcium, nearly 40% was in the pectate fraction (C_1) and 37% in the hydrochloric acid extract (C), so that, with the exception of iron and molybdenum more than 70% of each of the elements studied was extracted by ethanol and water. In this thesis, the total ^{51}Cr activity readily soluble, i.e. extracted by ethanol, water and hydrochloric acid, varied from 11 to 62%, but was greater than 30% for most samples. Furthermore, relatively little ^{51}Cr radioactivity was found in separations for nucleic acids, pectates or proteins, while Bowen et al (1962) found only calcium in pectates and none of the other elements in these fractions.

The chemical form of chromium in plants was not able to be ascertained directly by the extraction procedure. In these investigations, the ethanol- and water-soluble ^{51}Cr obtained from clover, H. odora and manuka, were studied by high-voltage electrophoresis. Invariably the electrophoretically mobile ^{51}Cr was found as anionic species at pH 5.3 in pyridine/acetic acid buffer. This activity was usually resolved into one or more distinct peaks.

The ethanol extract of roots of nutrient-grown manuka plants contained ^{51}Cr as three distinct compounds, with the greatest part of the activity present as the most mobile compound which was identified as the trioxalatochromate(III) ion. This is unlikely to be an artifact of the ethanol extraction procedure since preparation of synthetic potassium trioxalatochromate(III) required much stronger conditions, viz. addition of solid potassium dichromate to saturated oxalic acid at 70°C followed by addition of excess dipotassium oxalate and heating at 100°C. Mixing of 0.1M sodium ^{51}Cr-chromate with oxalic acid at room temperature did not form labelled complex. Once formed, this complex is not very labile ($k = 2 \times 10^{-3}$ Moles min^{-1} at 40°C, 0.2M $HClO_4$) (Banerjea and Mohan, 1963), with the rate of dissociation becoming very small in dilute solution at normal temperatures and in the absence of acid.

The presence of chromium as the oxalate complex, after feeding of the plant with chromate, means that chromium was reduced in the plant. Donaldson and Barreras (1966) found that, although orally administered $Na_2{}^{51}CrO_4$ and $^{51}CrCl_3$ were not absorbed appreciably by humans, the in vitro intestinal uptake of $Na_2{}^{51}CrO_4$ was greater than that of $^{51}CrCl_3$. They also showed that Cr(VI) was reduced by acid gastric juices to Cr(III) which was poorly absorbed. Schroeder (1968)

considered that chromium was essential for mammals and involved in lipid and glucose metabolism but its state of binding or mode of interaction has not been elucidated. In an earlier paper, Schroeder et al (1962) reported that from three-eighths (in oak leaves) to seven-eighths (in tomatoes) of the chromium found in ash of various plant and animal tissues was in the Cr(III) state, the remainder as Cr(VI).

Pierce (1964) has presented evidence for the binding of ^{51}Cr with human serum proteins, and has shown the specific involvement of carboxylic groups. In the yeast Saccharomyces carlsbergensis an unidentified low molecular weight complex has been reported when this organism was cultured in the presence of Cr(III) (Burkeholder and Mertz, 1966), but this thesis reports the first account of a known chromium complex in plants.

Cobalt-60 and ^{65}Zn extracted by ethanol from tomato leaves have been studied chromatographically by Bowen et al (1962) but ^{60}Co was neither the divalent cation nor as Vitamin B_{12}. Zinc, though partly as Zn^{2+} was also present in another compound. A soluble zinc complex has also been isolated from leaves of the grass Agrostis tenuis which had been fed with ^{65}Zn (Peterson, 1969). By gel filtration, Wilson and Nicholas (1967) have also shown that ^{60}Co in sterile cultures of clover (Trifolium subterraneum) was not as either the inorganic ion nor Vitamin B_{12} (cobalamin). Complexes of organic acids with iron have been found in exudates of tomato and sunflower (Tiffen 1966a, 1966b, 1967). Iron in tomato occurred as a complex with citrate (Tiffen, 1967) but manganese, cobalt and zinc were cations as determined by electrophoresis.

The trioxalatochromate(III) complex has a dissociation constant ($\log K_3 = 5.47$) which is nearly an order of magnitude greater than the corresponding iron-oxalate complex ($\log K_3 = 4.77$) (Sillen and Martell, 1964) indicating a higher degree of stability for the chromium complex. Although both such complexes would tend to dissociate in solution unless there was a high concentration of oxalic acid also present, the chromium complex would do so much slower than iron or most other metal complexes (Cotton and Wilkinson, 1962, p. 548). There is an extreme scarcity of data on the stability of metal ions with organic acids in the compilation of Sillen and Martell (1964) but undoubtably competition between chromium and ions such as calcium, magnesium, iron and manganese, for organic acids will occur, as with citrate (Tiffen, 1967).

The results from the xylem sap experiments show that hexavalent chromate is absorbed from solution and transported in the xylem throughout the manuka plant. Metabolism of chromate would therefore take place in the leaf tissue to give trioxalatochromate(III). Whether or not metabolism of $^{51}CrO_4^{2-}$ to the chromium complexes takes place only in the leaves and is then translocated in the phloem to the rest of the plant or whether all tissues can synthesise the complexes remains to be ascertained.

The transport of chromium as chromate in the xylem sap of manuka is therefore analogous to sulphur and phosphorus which are principally transported as inorganic ions in xylem sap (Tolbert and Wiebe, 1955). The results of this study contrast with the work of Tiffen (1966a, 1966b) who showed that iron was transported as a complex with citric acid in the xylem sap of several species.

PART V

GENERAL DISCUSSION

From the study undertaken, and reported in this thesis, it has been shown that the biogeochemical method of prospecting could be implemented successfully in New Zealand. In order, however, to assess its actual importance in the New Zealand environment, it should be compared critically with other methods which are available.

For geochemical prospecting, for broad surveys stream sediment and water analyses can give some localisation of areas of mineralisation, but for more particular localisation, either soil or biogeochemical analysis must be carried out. A comprehensive comparison of these two methods is not possible from the limited results presented in this thesis, but general comments can be made.

Sampling of soil in New Zealand's dense bush may often be much more difficult than sampling plants, since the soil is often concealed by undergrowth and tangled root systems may prevent access to the lower horizons. However, this was the case at only a few of the sampling sites at Copperstain Creek. It is, though, often difficult to identify a particular horizon on poorly-differentiated soils, and an arbitrary sampling depth must be used. A further advantage of the biogeochemical method, is that leaf samples weigh considerably less than the amount of soil required to obtain a representative sample.

Biogeochemical sampling is easier than soil sampling in terms of effort and labour, but involves more skill than soil sampling. Plant samples must be collected from the correct species which may not always be easily recognised, and the correct part and age of plant must be selected. This then, introduces the major disadvantage of the biogeochemical method: the need for an orientation survey. In each area, the most suitable plant species must be found, since

some species do not indicate mineralisation. In general, however, comprehensive soil surveys should be made only after an introductory study has been made, but for soil sampling, there are fewer variables to be considered than there are for biogeochemistry.

In the particular case of the Copperstain Creek prospect, molybdenum mineralisation was able to be indicated by the analysis of leaf ash of Olearia rani. The other two species were not able to give such a reliable estimate of the molybdenum anomaly. For copper, soil geochemical analysis again was able to indicate the presence of this element, but none of the tree plant species considered in this thesis, when analysed for copper, showed any indication of the extent or degree of copper mineralisation. However, this survey may not have been a fair test of the biogeochemical method, as the mineralisation has been shown to be of low grade and limited extent so that mining is not an economic proposition, even for sulphur, which was present at an average content of about 7% in the underlying rocks. Such a large sulphur content could affect the accumulation of such metals as copper also, but to what extent is not known.

The suitability of several species of plant from the Mineral Belt, for biogeochemical prospecting, has been shown. In particular Cassinia vauvilliersii shows high degrees of correlation between the plant ash and soil concentrations of chromium, nickel and cobalt. Leptospermum scoparium, Hebe odora and Pimelea suteri also show that analyses of their leaves for chromium could indicate the presence of high chromium contents in the underlying soil.

Strictly, the serpentine plants will probably only be able to indicate mineralisation with a high degree of reliability in serpentine soils. However, on pure serpentine or peridotite areas,

such as Dun Mountain, the vegetation is so sparse, and outcrops so prolific, that field geology would usually be sufficient to find mineral deposits, and biogeochemistry will be of limited assistance only. However, the common plant species may prove useful in the search for nickel or chromium in alpine areas of basic rocks where serpentisation has not occurred to the degree of modifying the vegetation to the extent that has occurred on the Mineral Belt.

The use of statistical methods for biogeochemistry in this thesis represents an advance over the previous empirical methods which were used for determining whether a plant species is a good biogeochemical indicator or not. By calculation of the correlation coefficient between the metal contents of plant ash and soil, the statistical probability that a given plant ash content indicates a certain soil concentration can be estimated. However, to make reliable inferences from statistical results, relatively large numbers of samples should be used, since, with smaller sample sizes, more care would be needed in interpretation of the statistical data.

The plotting of graphs showing percentage cumulative frequency plotted against concentration can also prove useful. At Copperstain Creek, this method showed a suitable threshhold value for the molybdenum contents of O. rani samples, but again, this statistical method should be limited to applications involving only large numbers of samples.

In general, biogeochemists and geochemists could and should avail themselves of modern statistical methods for large sets of data. Assistance from statisticians in the design of experiments could also prove useful. The availability of computers for the

tedious calculations makes statistics much more readily available than previously.

During the course of these biogeochemical investigations, the use of emission spectrography was found to be more satisfactory than absorption spectrophotometry for trace element analysis. Emission spectrography produces a permanent record for the analysis of many elements simultaneously and, for soils in particular, sample preparation was simple. Only for zinc, calcium and magnesium analyses did the advantages of speed, sensitivity and precision of atomic absorption spectrophotometry make this method more preferable.

In addition to biogeochemistry, a considerable amount of data of general nutritional interest is recorded and discussed in this thesis. Trace element metabolism in plants in general is not well understood, and these results represent the first major accumulation of data for New Zealand indigenous plants and their corresponding soils.

The most interesting finding is that several plant species do, in fact, show highly-significant correlations between the metal content of the plant ash and the total concentration in the corresponding soil. The total soil content of a metal, as measured, is never all available to a plant and, therefore, these relationships indicate a high degree of correlation between the total soil content and what is available to the plant. In particular, the high accumulation of chromium from soils containing chromium in very insoluble forms is surprising. The ability of the plants to extract chromium from soil minerals, such as chromite, is much more efficient than is a 2.5% acetic acid solution, as nickel is more soluble than chromium in this extractant,

but is accumulated to a lower degree.

Selective accumulation of some elements by New Zealand indigenous flora has been described here. In particular, the serpentine endemic species Pimelea suteri has the ability to accumulate cobalt and nickel to far higher levels than the other plants studied. This plant contained mean concentrations of 5800 ppm nickel and 230 ppm cobalt in the ash which are respectively 90 and 30 times the average contents of these elements in plant ash (Hawkes and Webb, 1962). This degree of accumulation cannot be explained on the basis of present knowledge, as these elements are believed to be non-essential to plants, as is the element chromium also. Chromium occurred in all the serpentine plants, and was accumulated in particular by P. suteri and manuka.

The serpentine plants differed from plants sampled on normal soils and differed between species, not only in their trace element content, but also in their calcium and magnesium contents, and the balance of these two elements. As with the apparent selectivity of trace elements by different species, it is not known whether the plants have different requirements for the various elements, or it may be that different species operate different mechanisms to increase or restrict the uptake of these elements.

The factors influencing the accumulation of any element by plants are many and complex. Soil factors such as drainage, porosity and particle size and site factors such as aspect and elevation may affect the growth and mineral accumulation of plants as well as chemical factors of pH and nutrient status of the soil. Interactions may occur in the soil and in the plant, between major and minor elements and each other, to give the final elemental composition of the plant.

For trace elements in particular, interactions with other trace elements can be important, but are difficult to evaluate. Linear correlations were calculated between pairs of elements in the plant species sampled from serpentine soils and in the soils themselves and showed a number of significant relationships.

It was found that in the soil chromium, nickel and cobalt increase in concentration simultaneously, and copper also but to a lesser extent. When considering the trace element nutrient status of serpentine soils it therefore is impossible to determine any effect due to the presence of unusually high amounts of any one of these elements, as in the same soil, the other three will also occur at high total contents.

In the plants from the Mineral Belt, the foliar contents of the six elements of particular interest, chromium, nickel, copper, cobalt, calcium and magnesium, when considered for linear correlations between any two, showed relationships of varying significance, depending on the species and the sample population considered. In general, it can be said that if the concentration of, say, chromium, is higher in one plant than in another (of the same species) then the concentrations of nickel, cobalt and magnesium will also be higher and that of calcium lower. These are the same general characteristics as those of soils derived from a substrate varying from sedimentary rocks to ultramafic.

The overall interdependence of the various factors, one on another, would be very difficult to determine. The use of linear correlations in this thesis has yielded a certain amount of information but more could probably be obtained by use of more sophisticated statistical techniques. In a recent thesis on the phosphate status of soils,

Ballard (1968) was able to show which soil and site factors had a direct effect and which an indirect effect on the productivity of Pinus radiata, by the use of multiple and partial correlations calculated with a computer. A similar type of study could be done on serpentine plants, but a trained statistician would be required to interpret the results.

These studies showed that the New Zealand serpentine mineral environment is complex and, as with overseas studies (e.g. Sarosiek, 1964), the indigenous plants showed as a direct response to the limiting factors of this environment, changes in the foliar elemental composition. However, it has not been resolved which are the most important factors. To solve this question would require complex, well designed growth experiments.

Experiments should be carried out initially to study at least three species: a serpentine endemic (e.g. P. suteri), a "serpentine-adapted" species such as manuka (Leptospermum scoparium), and a non-tolerant species such as red beech (Nothofagus fusca). By careful design, a series of nutrient culture solutions could be prepared, ideally to contain a range of concentrations of the elements of particular interest in serpentine soils, viz. Cr, Ni, Cu, Co, Ca, Mg, Mo, Fe, N, P and K. If plants were cultured under identical conditions of external climate, and measurements made on the degree of germination, survival and growth, then with suitable statistical analysis a large amount of information on the reasons for development of a peculiar serpentine flora could be deduced. However, such a large experiment must, because of its size, be a long term project.

Further information could be obtained by complete mineral analyses of the plants from such growth experiments, with further

statistical analysis. Possibly also, the effect of mineralisation on morphological characteristics could be determined by examination of the same plants.

Following on from the study of the contents of trace elements in plants, an attempt was made to understand something of the metabolism of chromium, an element which occurred at unusually high concentrations in serpentine plants. Using the radioisotope ^{51}Cr, the trioxalatochromate(III) ion was identified in roots and shoots of manuka seedlings, but not in red clover seedlings. In xylem sap, however, chromium was present as the inorganic ion ^{51}Cr-chromate. Other soluble compounds of chromium were separated from plant extracts, but have yet to be identified.

The chemical composition of the chromium containing components of both manuka and red clover seedlings could be further characterised by the isolation of larger amounts of these compounds, which may be able to be identified by mass spectrometry. This technique can now be used often on very small samples, to identify the chelate fragments of metal complexes (R.J. Hodges, pers. comm.). Useful comparisons could also be made between plants grown under identical conditions from seed from both a normal environment and the serpentine area.

Overall, this radioisotope study has shown some aspects of chromium metabolism, and differences between indigenous and pasture plant species. The tracer isotope method should be used more, and could give further insights into the metabolism and the mode and rate of movement of chromium and other inorganic ions, with the eventual aim of understanding how plants can and do indicate mineralisation. This information will be necessary to place biogeochemical prospecting on a more secure footing from the mineral metabolism viewpoint.

This would obviate the deficiencies in the past when the technique has been applied in an essentially empirical manner with little thought to plant physiology or to statistical considerations.

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APPENDICES

Appendix 1a

GRID SURVEY RESULTS FROM COPPERSTAIN CREEK

(ppm of ash)

(Hyphen indicates plant absent at site. pH was not measured on all soils)

Site No.	Soil				Olearia rani			Myrsine salicina		Quintinia acutifolia	
	pH	Mo	Cu	Zn	Mo	Cu	Zn	Mo	Cu	Mo	Cu
12	5.0	35	185	70	10	170	1250	15	90	-	-
13	6.0	98	190	165	5	130	580	-	-	9	80
14	5.5	62	180	185	5	110	580	6	70	13	76
15	5.5	160	260	120	-	-	-	-	-	3	60
22	5.3	145	135	80	15	330	520	20	64	13	170
23	5.2	96	145	50	200	180	1640	50	62	90	100
24	5.3	135	185	95	18	125	960	25	65	-	-
25	5.4	155	200	120	44	115	500	7	80	-	-
26	4.9	155	165	55	-	-	-	-	-	12	64
31	4.9	125	185	95	64	115	420	7	100	-	-
32	5.3	90	120	75	74	145	1220	16	70	-	-
33	5.5	215	92	55	100	250	900	9	74	70	105
34	5.5	110	175	100	66	230	820	6	88	-	-
35	5.4	210	80	130	92	290	960	6	76	-	-
36	4.8	195	73	30	-	-	-	20	52	230	115
41	6.8	100	125	185	40	500	450	-	-	-	-
42	5.8	135	220	90	110	250	850	-	-	12	145
43	5.7	105	105	40	72	360	660	-	-	230	90
44	5.1	170	70	75	260	840	1000	66	140	190	250
45	5.5	130	68	80	20	205	590	5	92	-	-
46	5.6	125	60	80	112	350	660	-	-	33	130
47	5.1	160	72	60	-	-	-	18	115	9	160
51	5.3	41	110	110	3	360	600	5	135	-	-
52	5.3	90	71	80	22	250	500	-	-	-	-
53	5.4	155	190	60	26	490	320	-	-	-	-
54	5.8	130	83	90	-	-	-	44	52	100	230
55	5.2	135	50	75	80	510	920	30	150	24	300
56	5.2	145	73	95	-	-	-	-	-	10	200
57	6.0	215	72	45	50	370	470	-	-	-	-
58	5.4	215	280	125	28	220	830	51	92	33	130
59	5.2	125	280	80	-	-	-	-	-	9	130

cont.

Appendix 1a (continued)

Site No.	pH	Mo	Cu	Zn	Mo	Cu	Zn	Mo	Cu	Mo	Cu
61	5.8	54	108	210	7	70	280	10	70	-	-
63	5.3	80	62	60	62	360	460	20	56	7	180
64	5.3	75	88	55	-	-	-	38	130	23	220
65	5.1	130	120	115	13	25	700	-	-	-	-
66	5.4	115	42	125	70	180	580	13	90	-	-
67	5.4	115	105	85	30	305	830	5	105	-	-
68	4.9	60	42	35	-	-	-	80	100	9	130
71	5.1	150	100	60	74	240	1130	13	62	-	-
72	5.5	100	80	60	18	74	470	-	-	-	-
73	5.3	36	160	70	7	160	720	-	-	9	170
75		64	90	140	9	400	580	2	115	1	125
76		68	74	50	40	160	770	-	-	150	200
77		160	380	40	-	-	-	-	-	250	80
78		180	220	5	-	-	-	-	-	5	115
81		135	80	60	-	-	-	2	56	1	72
82		55	62	30	5	230	470	-	-	5	160
83		23	105	65	1	220	500	1	74	-	-
84		21	95	50	-	-	-	13	56	5	74
85		30	70	30	1	120	380	2	80	3	190
86		50	102	80	4	125	420	6	95	3	150
87		200	180	55	10	190	930	-	-	9	155
91		30	60	45	3	270	390	-	-	6	150
92		16	110	20	3	170	400	2	100	4	175
93		13	140	90	-	-	-	20	115	10	116
94		9	190	90	3	220	700	12	105	7	140
95		12	48	55	-	-	-	5	90	7	170
96		12	70	45	-	-	-	1	94	5	150
97		18	60	55	-	-	-	1	46	5	140
101		16	130	110	1	140	520	7	100	9	210
102		16	70	170	5	260	420	6	210	7	500
104		28	105	125	6	190	500	3	95	6	320
105		17	44	55	-	-	-	-	-	9	130
106		30	32	75	-	-	-	5	140	13	160

Appendix 1b

ANALYSIS OF OLEARIA RANI

(ppm of ash)

Leaves			Wood			Twigs			Flowers			Soil			Percentage Ash			
Mo	Cu	Zn	Mo	Cu	Zn	Mo	Cu	Zn	Mo	Cu	Zn	Mo	Cu	Zn	L	W	T	F
53	160	450	17	-	-	95	230	660	-	-	-	255	200	88	7.7	1.8	23.0	7.9
1600	140	720	210	480	1060	95	590	1000	-	-	-	200	180	94	7.7	1.4	7.9	7.9
200	140	1340	84	290	700	110	710	660	20	190	480	250	110	73	7.7	1.4	3.0	7.2
130	120	780	14	160	1300	50	310	1000	7	170	310	260	280	88	8.2	1.9	7.7	7.9
100	140	1230	70	310	1780	28	100	700	34	170	400	150	100	80	7.7	1.6	11.0	8.0
49	190	660	13	230	530	25	60	240	22	230	450	240	130	130	7.3	1.9	9.6	7.6
95	310	900	71	460	560	27	540	480	60	360	480	168	170	110	8.0	1.6	5.1	7.5
70	140	800	27	70	600	25	160	330	27	220	280	180	140	170	8.1	3.9	6.1	8.1
21	90	940	20	190	800	26	380	840	35	240	400	280	360	148	9.1	2.0	4.8	7.5
27	120	840	24	270	660	20	90	430	70	220	400	235	180	188	8.7	1.5	15.3	7.5
12	150	720	15	310	900	21	260	900	22	220	400	250	320	94	9.0	1.9	8.8	8.3
10	140	700	14	260	430	15	500	750	18	240	360	265	360	94	7.7	1.2	6.5	6.9
55	140	720	32	380	570	44	130	520	72	190	300	215	160	73	8.0	1.8	10.5	8.2
270	110	640	10	180	430	84	300	670	49	210	380	520	100	73	6.9	1.7	5.0	7.3
1000	220	910	110	310	720	145	690	830	50	270	430	330	170	135	5.9	1.5	6.3	8.1
150	130	640	66	360	370	58	360	570	70	250	370	350	280	120	5.9	2.0	5.7	6.5
82	130	450	6	300	380	3	380	600	4	180	250	205	130	70	6.9	1.3	4.9	7.9
34	130	430	30	340	480	37	570	390	26	190	240	180	120	73	7.5	1.8	6.1	8.2
70	170	550	10	170	450	13	840	430	16	190	220	270	410	188	6.7	1.8	4.6	8.1
33	180	720	15	220	520	19	330	800	6	220	300	120	280	130	8.9	1.4	5.9	8.3
110	220	640	44	280	700	60	480	780	55	190	300	155	140	94	5.9	1.7	5.4	8.0
10	60	340	16	110	240	12	250	380	20	160	260	84	120	203	8.6	1.3	5.0	8.8
19	130	450	23	160	520	23	210	570	40	210	380	235	320	94	9.9	1.8	8.4	8.1
90	160	780	35	190	720	13	570	480	6	160	240	150	220	81	5.7	1.1	2.8	7.1
10	90	360	7	140	430	14	190	280	23	140	180	72	110	144	8.6	2.6	7.4	9.3
10	160	700	3	160	550	9	310	480	8	220	300	58	100	135	7.6	1.8	6.8	8.0

APPENDIX 2a

CASSINIA VAUVILLIERSII VAR. SERPENTINA

RANDOM COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
1400.0	27000.0	1200.0	4300.0	185.0	270.0	240.0	700.0	450.0	10.90	7.10	1.535
2300.0	11000.0	1400.0	1600.0	47.0	55.0	56.0	310.0	730.0	19.60	2.70	7.259
3400.0	16000.0	980.0	2800.0	325.0	119.0	70.0	280.0	350.0	4.40	9.70	.453
1000.0	28000.0	720.0	2400.0	90.0	124.0	25.0	310.0	470.0	11.20	5.00	2.240
4400.0	33000.0	1300.0	2200.0	21.0	63.0	76.0	300.0	800.0	8.20	7.40	1.108
900.0	44000.0	520.0	2500.0	11.0	96.0	34.0	300.0	630.0	5.00	4.50	1.111
6000.0	23000.0	1400.0	3000.0	33.0	125.0	100.0	330.0	480.0	10.60	7.10	1.492
130.0	5000.0	2500.0	2900.0	215.0	43.0	150.0	300.0	500.0	7.30	16.30	.447
520.0	5600.0	1000.0	3100.0	200.0	47.0	33.0	330.0	680.0	13.40	10.10	1.326
960.0	4600.0	1350.0	2200.0	180.0	117.0	67.0	240.0	440.0	9.00	7.90	1.139
1800.0	6800.0	2200.0	2900.0	160.0	155.0	50.0	910.0	670.0	11.80	5.60	2.107
1000.0	4700.0	3000.0	4800.0	155.0	85.0	71.0	580.0	460.0	4.30	12.50	.344
1050.0	3800.0	4000.0	4800.0	250.0	162.0	210.0	460.0	650.0	6.40	16.70	.383

APPENDIX 2b

CASSINIA VAUVILLIERSII VAR. SERPENTINA

LOCALISED COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
35.0	9000.0	660.0	2200.0	190.0	58.0	26.0	410.0	550.0	8.50	2.80	3.035
800.0	8000.0	1100.0	1850.0	36.0	66.0	40.0	320.0	470.0	8.80	8.00	1.100
1600.0	12000.0	1800.0	1900.0	300.0	60.0	42.0	400.0	1400.0	12.20	5.50	2.218
2500.0	11000.0	2000.0	1600.0	135.0	51.0	100.0	380.0	930.0	6.40	4.40	1.454
145.0	11000.0	750.0	3100.0	84.0	44.0	19.0	630.0	960.0	9.80	4.60	2.130
4000.0	11000.0	1800.0	1800.0	200.0	80.0	110.0	400.0	630.0	5.60	4.80	1.166
180.0	10500.0	1000.0	2000.0	84.0	52.0	34.0	480.0	780.0	6.50	4.00	1.625
1500.0	10500.0	1700.0	1900.0	230.0	91.0	85.0	410.0	800.0	10.60	11.40	.929
1100.0	11000.0	2300.0	2700.0	150.0	72.0	130.0	480.0	540.0	5.60	7.20	.777
2400.0	7300.0	1900.0	1900.0	260.0	81.0	56.0	300.0	760.0	4.70	4.50	1.044
1500.0	6800.0	1700.0	2150.0	155.0	64.0	80.0	360.0	760.0	7.00	9.00	.777
2100.0	7300.0	1850.0	1800.0	290.0	66.0	74.0	310.0	710.0	7.90	7.40	1.067
1900.0	6500.0	1450.0	2150.0	68.0	64.0	77.0	380.0	680.0	5.10	6.80	.750
1050.0	14000.0	880.0	1900.0	66.0	56.0	44.0	430.0	860.0	11.40	4.30	2.651
160.0	12000.0	700.0	2200.0	95.0	78.0	23.0	350.0	1240.0	11.10	6.40	1.734

APPENDIX 2c

CASSINIA VAUVILLIERSII VAR. SERPENTINA

BOUNDARY COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
41.0	860.0	220.0	1450.0	60.0	88.0	15.0	115.0	610.0	13.30	3.60	3.694
33.0	1250.0	105.0	1150.0	66.0	70.0	19.0	115.0	540.0	10.00	2.70	3.703
20.0	2700.0	240.0	1600.0	58.0	105.0	7.0	200.0	430.0	11.70	1.70	6.882
3.0	1900.0	165.0	920.0	66.0	86.0	7.0	150.0	580.0	13.40	.80	16.750
4.0	1600.0	101.0	980.0	53.0	67.0	4.0	140.0	330.0	8.40	1.60	5.250
20.0	3300.0	230.0	1000.0	110.0	90.0	14.0	200.0	440.0	10.40	.80	13.000
<10.0	4100.0	250.0	1500.0	220.0	70.0	70.0	205.0	620.0	10.30	1.90	5.421
<10.0	3700.0	600.0	1300.0	150.0	63.0	41.0	210.0	500.0	10.10	2.20	4.590
<10.0	2200.0	190.0	1040.0	100.0	74.0	5.0	150.0	500.0	12.70	1.50	8.466
<10.0	2100.0	560.0	1250.0	74.0	150.0	10.0	140.0	670.0	7.80	1.50	5.200

APPENDIX 2d

CASSINIA VAUVILLIERSII VAR. SERPENTINA

SAMPLES FOR POLLEN ANALYSIS

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
7200.0	90000.0	420.0	2700.0	55.0	135.0	30.0	360.0	610.0	24.90	2.90	8.586
130.0	23000.0	400.0	5600.0	32.0	200.0	11.0	710.0	660.0	18.40	2.60	7.076
4100.0	20000.0	700.0	2500.0	99.0	105.0	32.0	270.0	480.0	4.20	8.00	.525
540.0	2000.0	830.0	3800.0	45.0	140.0	66.0	390.0	850.0	5.90	4.60	1.282
<10.0	1600.0	250.0	1400.0	91.0	340.0	15.0	140.0	680.0	7.80	1.40	5.571
<10.0	900.0	140.0	710.0	53.0	96.0	10.0	83.0	830.0	9.50	1.30	7.307

APPENDIX 2e

HEBE ODORA

RANDOM COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
370.0	19000.0	2100.0	5900.0	200.0	400.0	36.0	760.0	370.0	10.50	12.40	.846
76.0	14000.0	1300.0	4000.0	130.0	250.0	29.0	580.0	470.0	10.50	6.70	1.567
3000.0	16000.0	2200.0	2800.0	190.0	119.0	160.0	280.0	410.0	4.60	24.90	.184
1700.0	35000.0	1050.0	1900.0	240.0	175.0	44.0	320.0	590.0	5.70	13.20	.431
1200.0	25000.0	780.0	2400.0	215.0	120.0	40.0	290.0	740.0	8.70	14.90	.583
920.0	44000.0	5600.0	2500.0	220.0	96.0	17.0	300.0	630.0	4.60	14.40	.319
1500.0	23000.0	1200.0	3000.0	245.0	125.0	33.0	330.0	530.0	6.30	20.60	.305
135.0	14000.0	2100.0	3400.0	160.0	155.0	33.0	500.0	720.0	7.80	14.10	.553
160.0	17000.0	1900.0	4300.0	79.0	185.0	34.0	580.0	600.0	6.50	16.30	.398
50.0	4500.0	1300.0	1600.0	95.0	79.0	40.0	130.0	290.0	10.30	8.10	1.271
520.0	7400.0	1900.0	1600.0	105.0	125.0	50.0	200.0	580.0	13.00	11.40	1.140
100.0	5300.0	1400.0	3700.0	120.0	69.0	35.0	530.0	600.0	7.90	10.20	.774
50.0	4200.0	2000.0	3300.0	160.0	75.0	39.0	460.0	350.0	5.90	12.60	.468

APPENDIX 2f

HEBE ODORA

LOCALISED COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
780.0	6200.0	1550.0	1300.0	107.0	67.0	33.0	250.0	540.0	8.80	14.20	.619
135.0	14500.0	2000.0	1600.0	210.0	87.0	20.0	490.0	890.0	11.60	7.60	1.526
190.0	11000.0	1800.0	2000.0	140.0	105.0	25.0	350.0	530.0	8.40	17.50	.480
52.0	7000.0	2400.0	1900.0	170.0	72.0	25.0	410.0	450.0	8.70	9.30	.935
2000.0	11500.0	2900.0	1550.0	250.0	81.0	74.0	400.0	470.0	5.90	8.80	.670
50.0	7000.0	3500.0	2100.0	225.0	95.0	34.0	400.0	780.0	9.40	16.80	.559
72.0	10300.0	1500.0	2300.0	175.0	81.0	29.0	430.0	540.0	7.00	16.30	.429
230.0	7000.0	1600.0	2600.0	155.0	150.0	22.0	520.0	880.0	7.50	14.00	.535
48.0	8400.0	1900.0	3000.0	140.0	120.0	31.0	660.0	630.0	8.50	23.10	.367
300.0	12000.0	1300.0	1800.0	55.0	87.0	31.0	520.0	770.0	10.50	11.80	.889
1200.0	9000.0	1850.0	1900.0	150.0	76.0	32.0	480.0	590.0	7.10	19.30	.367
52.0	7200.0	1200.0	1650.0	150.0	76.0	29.0	330.0	630.0	9.90	17.40	.568
44.0	6800.0	1450.0	2150.0	295.0	115.0	18.0	360.0	630.0	6.70	6.10	1.098
37.0	5900.0	1400.0	2600.0	200.0	97.0	19.0	480.0	580.0	6.30	13.40	.470
76.0	8200.0	3700.0	2800.0	195.0	115.0	82.0	460.0	770.0	6.80	21.50	.316

APPENDIX 2g

HEBE ODORA

BOUNDARY COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
14.0	1400.0	540.0	1450.0	60.0	85.0	6.0	110.0	720.0	14.30	3.10	4.612
26.0	1600.0	350.0	980.0	60.0	67.0	12.0	140.0	510.0	14.70	3.20	4.593
17.0	3300.0	480.0	1000.0	80.0	90.0	15.0	200.0	380.0	11.90	3.10	3.838
<10.0	1000.0	440.0	1040.0	68.0	165.0	9.0	96.0	510.0	10.90	3.60	3.027
<10.0	960.0	620.0	1300.0	39.0	62.0	6.0	110.0	630.0	18.10	2.00	9.050
<10.0	1400.0	310.0	1400.0	64.0	130.0	45.0	125.0	580.0	9.30	3.10	3.000
<10.0	2200.0	420.0	1040.0	54.0	74.0	70.0	150.0	370.0	12.00	3.70	3.243
<10.0	2700.0	540.0	1600.0	145.0	105.0	70.0	200.0	630.0	13.50	3.10	4.354
<10.0	1900.0	330.0	920.0	72.0	86.0	16.0	150.0	420.0	14.50	3.60	4.027
<10.0	4100.0	500.0	1500.0	72.0	70.0	10.0	205.0	450.0	12.10	4.70	2.574

APPENDIX 2h

LEPTOSPERMUM SCOPARIUM

RANDOM COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
1800.0	7000.0	2300.0	5400.0	120.0	140.0	65.0	480.0	670.0	15.90	8.90	1.786
320.0	5600.0	1550.0	3200.0	59.0	107.0	33.0	330.0	590.0	27.50	8.30	3.313
920.0	6000.0	1550.0	3900.0	55.0	83.0	93.0	400.0	460.0	15.00	10.80	1.388
2500.0	2800.0	880.0	3200.0	83.0	77.0	34.0	250.0	390.0	18.70	8.20	2.280
19000.0	19000.0	13000.0	5900.0	200.0	400.0	400.0	750.0	310.0	11.20	7.40	1.513
10000.0	10000.0	9600.0	3500.0	115.0	215.0	420.0	540.0	310.0	12.10	8.30	1.457
2900.0	12000.0	740.0	3600.0	125.0	145.0	42.0	420.0	360.0	14.80	17.00	.870
7600.0	24000.0	2200.0	2400.0	130.0	120.0	105.0	280.0	330.0	12.60	12.00	1.050
10000.0	66000.0	2200.0	1950.0	95.0	75.0	107.0	320.0	430.0	14.30	12.00	1.191
10500.0	23000.0	2800.0	3000.0	210.0	125.0	160.0	330.0	340.0	14.00	8.60	1.627
390.0	15000.0	1700.0	3000.0	68.0	53.0	48.0	320.0	300.0	10.40	13.30	.781
200.0	5000.0	3100.0	2900.0	390.0	43.0	180.0	300.0	310.0	9.00	11.90	.756
1100.0	4600.0	2200.0	2200.0	75.0	117.0	40.0	240.0	430.0	11.50	12.30	.934
2900.0	6800.0	2500.0	2900.0	95.0	155.0	68.0	910.0	370.0	8.80	9.90	.888
4200.0	5300.0	7800.0	3700.0	75.0	69.0	460.0	530.0	350.0	8.90	6.70	1.328
3200.0	2600.0	2200.0	3900.0	57.0	75.0	53.0	540.0	320.0	16.00	6.80	2.352

APPENDIX 2i

LEPTOSPERMUM SCOPARIUM

LOCALISED COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
500.0	9000.0	1500.0	2200.0	79.0	58.0	40.0	410.0	560.0	14.30	7.40	1.932
5200.0	8000.0	2400.0	1850.0	43.0	66.0	145.0	320.0	390.0	13.10	10.00	1.310
2400.0	7000.0	2000.0	1900.0	47.0	72.0	58.0	410.0	390.0	19.40	6.30	3.079
2500.0	6000.0	1900.0	1900.0	99.0	61.0	76.0	310.0	440.0	22.90	4.30	5.325
1400.0	11000.0	1300.0	2700.0	87.0	60.0	41.0	500.0	440.0	17.90	12.10	1.479
700.0	6400.0	860.0	1750.0	50.0	79.0	25.0	360.0	340.0	22.00	6.40	3.437
1250.0	10600.0	1600.0	1900.0	82.0	69.0	35.0	430.0	460.0	16.30	8.40	1.940
1500.0	9800.0	1400.0	1300.0	125.0	48.0	46.0	290.0	490.0	25.70	6.20	4.145
5400.0	9600.0	3200.0	2000.0	185.0	96.0	290.0	420.0	530.0	15.70	7.20	2.180
6000.0	11000.0	5200.0	1900.0	120.0	69.0	170.0	400.0	410.0	19.20	5.40	3.555
1300.0	11000.0	1500.0	2700.0	90.0	72.0	42.0	480.0	470.0	22.00	11.20	1.964
8500.0	8400.0	2500.0	1600.0	75.0	63.0	200.0	370.0	350.0	15.20	6.80	2.235
800.0	6800.0	1100.0	2150.0	90.0	64.0	41.0	360.0	470.0	16.50	8.60	1.918
460.0	8400.0	2900.0	2000.0	250.0	96.0	170.0	390.0	450.0	19.60	7.90	2.481
1300.0	11000.0	540.0	1600.0	150.0	63.0	37.0	480.0	340.0	27.70	5.60	4.946

APPENDIX 2j

LEPTOSPERMUM SCOPARIUM

BOUNDARY COLLECTION

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
90.0	5200.0	110.0	1300.0	30.0	85.0	6.0	240.0	280.0	16.50	3.50	4.714
320.0	2700.0	840.0	1050.0	115.0	57.0	17.0	140.0	290.0	17.20	3.80	4.526
660.0	2200.0	1200.0	1050.0	120.0	73.0	40.0	130.0	270.0	18.60	5.90	3.152
170.0	2700.0	620.0	1500.0	60.0	82.0	20.0	225.0	350.0	17.30	4.50	3.844
160.0	1400.0	360.0	760.0	110.0	70.0	25.0	98.0	280.0	13.60	4.60	2.956
<10.0	3600.0	260.0	1500.0	76.0	84.0	50.0	200.0	220.0	19.00	3.20	5.937

APPENDIX 2k

LEPTOSPERMUM SCOPARIUM

SAMPLES FOR POLLEN ANALYSIS

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
75.0	13000.0	440.0	6400.0	41.0	110.0	8.0	480.0	530.0	14.50	5.10	2.843
1150.0	90000.0	420.0	2700.0	66.0	135.0	18.0	360.0	370.0	11.90	11.40	1.043
7200.0	25000.0	1900.0	3800.0	26.0	90.0	130.0	380.0	360.0	9.30	8.50	1.094
4100.0	10500.0	860.0	3100.0	48.0	125.0	56.0	330.0	350.0	11.30	9.60	1.177
850.0	5000.0	430.0	4700.0	46.0	180.0	24.0	460.0	530.0	7.60	12.80	.593
290.0	12000.0	380.0	6000.0	32.0	120.0	15.0	700.0	380.0	16.00	5.20	3.076
65.0	5400.0	200.0	2300.0	54.0	110.0	13.0	340.0	420.0	17.70	3.00	5.900
48.0	3700.0	100.0	1300.0	26.0	85.0	10.0	140.0	360.0	16.10	2.70	5.962
620.0	6200.0	1700.0	3400.0	50.0	78.0	62.0	310.0	590.0	8.20	14.60	.561
380.0	10500.0	630.0	1800.0	27.0	150.0	15.0	440.0	400.0	12.90	12.10	1.066
680.0	4400.0	740.0	4100.0	53.0	85.0	42.0	480.0	460.0	14.40	7.00	2.057

APPENDIX 21

MYOSOTIS MUNROI

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
540.0	13500.0	750.0	3200.0	50.0	92.0	38.0	600.0	170.0	5.00	3.40	1.470
190.0	5400.0	800.0	2200.0	60.0	125.0	36.0	240.0	180.0	4.30	2.70	1.592
130.0	6000.0	400.0	2300.0	30.0	155.0	29.0	230.0	190.0	3.70	1.80	2.055
640.0	24000.0	580.0	3250.0	45.0	100.0	36.0	530.0	300.0	3.50	2.40	1.458
390.0	6500.0	920.0	3000.0	61.0	19.0	45.0	460.0	210.0	4.10	1.50	2.733
2250.0	6700.0	800.0	3900.0	48.0	120.0	47.0	650.0	260.0	4.30	3.90	1.102
450.0	2600.0	750.0	2500.0	61.0	62.0	46.0	330.0	260.0	4.90	4.30	1.139
260.0	7300.0	660.0	2900.0	46.0	62.0	29.0	460.0	340.0	3.50	2.60	1.346
5000.0	6800.0	1100.0	2000.0	42.0	76.0	43.0	265.0	230.0	5.50	3.40	1.617
580.0	6500.0	540.0	2400.0	30.0	46.0	24.0	340.0	240.0	5.00	4.00	1.250
5500.0	18000.0	1100.0	2300.0	33.0	66.0	82.0	370.0	260.0	2.80	2.70	1.037
310.0	9500.0	1000.0	2800.0	38.0	82.0	29.0	390.0	260.0	2.30	2.30	1.000
5500.0	6500.0	2500.0	3500.0	105.0	145.0	165.0	460.0	230.0	4.00	2.90	1.379
1200.0	2900.0	1200.0	1600.0	58.0	330.0	50.0	155.0	260.0	5.30	2.60	2.038
155.0	3700.0	600.0	1650.0	41.0	100.0	32.0	180.0	230.0	4.10	2.00	2.050
300.0	13000.0	1650.0	2200.0	32.0	140.0	83.0	240.0	310.0	3.40	3.40	1.000
520.0	6800.0	800.0	3200.0	65.0	170.0	28.0	310.0	230.0	2.70	4.00	.675
2700.0	2500.0	400.0	2000.0	28.0	50.0	38.0	180.0	270.0	3.20	3.00	1.066
800.0	4400.0	1200.0	2200.0	39.0	270.0	56.0	280.0	260.0	2.90	3.70	.783
280.0	7600.0	1400.0	4100.0	39.0	310.0	35.0	460.0	260.0	3.00	3.20	.937

APPENDIX 2m

NOTOTHLASPI AUSTRALE

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
200.0	8600.0	640.0	3200.0	22.0	90.0	75.0	390.0	1300.0	5.00	15.30	.326
1350.0	14800.0	465.0	8000.0	18.0	210.0	66.0	840.0	910.0	5.50	15.10	.364
360.0	7900.0	435.0	2700.0	16.0	85.0	48.0	290.0	890.0	9.00	11.50	.782
800.0	29500.0	1000.0	5000.0	27.0	90.0	70.0	530.0	930.0	3.60	17.40	.206
265.0	5650.0	725.0	3200.0	35.0	90.0	56.0	360.0	1120.0	5.20	9.80	.530
330.0	8000.0	640.0	7500.0	24.0	150.0	160.0	650.0	1330.0	5.20	17.10	.304
35.0	8000.0	510.0	4800.0	8.2	95.0	65.0	420.0	650.0	4.70	12.50	.376
280.0	8600.0	580.0	3500.0	19.0	75.0	58.0	310.0	1000.0	4.70	10.10	.465
830.0	16000.0	1150.0	4200.0	23.0	115.0	125.0	690.0	960.0	5.40	14.00	.385
6900.0	24500.0	1050.0	6000.0	30.0	210.0	185.0	780.0	1000.0	4.00	11.00	.363
445.0	28300.0	810.0	3200.0	10.0	80.0	60.0	465.0	1520.0	4.00	14.30	.279
1390.0	27000.0	770.0	9000.0	27.0	340.0	70.0	735.0	-	-	-	-
530.0	11700.0	1520.0	2000.0	15.0	49.0	144.0	220.0	560.0	4.30	22.60	.190
400.0	6150.0	125.0	4100.0	14.0	49.0	165.0	205.0	870.0	11.90	9.30	1.279
160.0	18500.0	245.0	3500.0	6.8	94.0	60.0	500.0	1820.0	7.90	25.70	.307
2150.0	2950.0	1880.0	900.0	31.0	32.0	170.0	95.0	1400.0	5.60	17.00	.329
660.0	16000.0	540.0	3500.0	20.0	100.0	165.0	220.0	410.0	3.70	11.60	.318
1520.0	11600.0	580.0	3100.0	16.0	140.0	64.0	290.0	1250.0	4.80	15.00	.320
345.0	14800.0	670.0	4900.0	16.0	250.0	40.0	420.0	540.0	4.50	13.10	.343
230.0	24500.0	600.0	4900.0	25.0	160.0	46.0	360.0	1330.0	5.10	17.20	.296

APPENDIX 2n

PIMELEA SUTERI

CR (PPM)		NI (PPM)		CU (PPM)		CO (PPM)		ZN(PPM)	CA(%)	MG(%)	CA/MG
PLANT	SOIL	PLANT	SOIL	PLANT	SOIL	PLANT	SOIL		PLANT ASH		
1260.0	6400.0	7500.0	3500.0	105.0	100.0	360.0	360.0	770.0	4.70	11.80	.398
2800.0	7000.0	8550.0	1700.0	270.0	115.0	250.0	196.0	1700.0	9.20	18.00	.511
660.0	13600.0	3450.0	9000.0	80.0	440.0	104.0	840.0	1320.0	3.70	13.80	.268
1220.0	3300.0	8550.0	4800.0	130.0	100.0	56.0	390.0	1190.0	8.70	16.70	.520
230.0	13500.0	3900.0	4000.0	100.0	400.0	132.0	310.0	700.0	8.20	12.40	.661
1170.0	9800.0	5800.0	3800.0	70.0	92.0	470.0	390.0	940.0	7.50	14.70	.510
3950.0	11000.0	11500.0	4800.0	230.0	150.0	160.0	390.0	500.0	5.80	16.90	.343
380.0	8300.0	3300.0	2400.0	92.0	92.0	340.0	140.0	480.0	3.50	18.80	.186
11400.0	32000.0	10500.0	2400.0	200.0	270.0	480.0	125.0	600.0	5.40	20.00	.270
11600.0	28200.0	10000.0	2500.0	300.0	86.0	320.0	170.0	750.0	4.70	16.30	.288
330.0	13600.0	2300.0	5100.0	140.0	180.0	124.0	360.0	860.0	4.70	20.20	.232
890.0	4900.0	4900.0	3500.0	78.0	120.0	180.0	235.0	1470.0	4.90	15.20	.322
1340.0	5400.0	5500.0	3000.0	110.0	120.0	240.0	210.0	620.0	3.00	16.30	.184
890.0	6150.0	7250.0	2810.0	125.0	180.0	250.0	430.0	660.0	3.60	19.80	.181
680.0	9800.0	4200.0	2700.0	86.0	130.0	240.0	265.0	680.0	3.00	15.10	.198
26500.0	56500.0	10000.0	2400.0	200.0	130.0	540.0	170.0	750.0	6.80	10.50	.647
3800.0	31000.0	4500.0	2600.0	84.0	100.0	220.0	190.0	720.0	6.30	9.50	.663
4100.0	17000.0	13000.0	3200.0	200.0	125.0	600.0	240.0	540.0	5.30	15.50	.341
2300.0	29500.0	2040.0	1900.0	160.0	100.0	145.0	115.0	600.0	3.00	23.20	.129
3470.0	18500.0	5800.0	7250.0	62.0	410.0	280.0	670.0	-	-	-	-

Appendix 3

ANALYSES OF SOME PLANTS USED FOR CHROMIUM-51 STUDIES

(As referred to in Part IV)

Sample	Ash Content (% dry wt.)	Element Content (ppm in ash) Cr	Ni	Cu	Co
Red clover grown in nutrient solution : shoots	8.7	<15	<15	250	16
Red clover grown in nutrient solution : roots	10.7	<10	<10	20	10
Manuka grown in nutrient solution : leaves	9.8	<10	40	160	12
Manuka grown in nutrient solution : stem	4.2	<10	<10	95	10
Manuka grown in nutrient solution : roots	5.0	20	20	135	10
H. odora grown in serpentine soil : roots	7.6	7400	3700	250	260
H. odora grown in Manawatu soil : roots	5.5	3500	1150	360	65
Manuka grown in serpentine soil : roots	17.6	4400	3700	150	290
Manuka grown in Manawatu soil : roots	7.6	450	550	230	60

Appendix 4

Computer programmes for calculation of correlation coefficients.

Note: These programmes were written for a IBM 1620(II) computer in the PDQ Fortran language, initially for the computer with 20k storage and later (Appendix 4c) for 40k storage. Programmes were sometimes used for purposes outside their original design by making simple modifications of the input data and corresponding allowances when reading the output information.

Appendix 4a

```
C      CORRELATION OF LOG-LOG DATA
C      USE PDQ FORTRAN CLC2 PROCESSOR
C      USE PDQ FIXED FORMAT SUBROUTINES
C      SENSE SWITCH 2 ON PRINTS INPUT DATA
    8  AN=0.0
       SX=0.0
       SY=0.0
       SXSQ=0.0
       SYSQ=0.0
       SXY=0.0
       READ 10
       PRINT 10
   10  FORMAT(40H
       IF (SENSE SWITCH 2)11,1
   11  PRINT 12
   12  FORMAT(/10X,1HA,10X,1HB/)
    1  READ 2,A,B,M
    2  FORMAT (2F6.0,67X,I1)
       IF(M-9)3,4,4
    3  AN=AN+1.0
       IF(SENSE SWITCH 2)14,16
   14  PRINT 15,A,B
   15  FORMAT(5X,2F10.3)
   16  X=0.43429448*LOGF(A)
       Y=0.43429448*LOGF(B)
       SX=SX+X
       SY=SY+Y
       SXSQ=SXSQ+X*X
       SYSQ=SYSQ+Y*Y
       SXY=SXY+X*Y
       GO TO 1
    4  GMX=EXPF(SX/ (AN*0.43429448))
    1  GMY=EXPF(SY/(AN*0.43429448))
    2  SDX=SQRTF((SXSQ-SX*SX/AN)/(AN-1.0))
    1  SDY=SQRTF((SYSQ-SY*SY/AN)/(AN-1.0))
    2  R=(SXY-SX*SY/AN)/(SQRTF((SXSQ-SX*SX/AN)*(SYSQ-SY*SY/AN)))
       RMA=SDX/SDY
    3  PRINT 5,AN
    5  FORMAT(// 18HNUMBER OF SAMPLES=,F6.0)
    4  PRINT 6,GMX,SDX
    6  FORMAT(/14HAVERAGE OF A =,F12.4,10X,10HSTD. DEV.=,F10.6)
    4  PRINT 9,GMY,SDY
    9  FORMAT(/14HAVERAGE OF B =,F12.4,10X,10HSTD. DEV.=,F10.6)
       PRINT 7,R,RMA
    7  FORMAT(//3HR =,F8.4,10X,18HRMA SLOPE (A/B) = ,F8.4)
       PAUSE
       GO TO 8
C      MEANS IN ORIGINAL UNITS, SD S IN LOG UNITS
       END
```

Appendix 4b

```
C       CORRELATION OF LOG-NORMAL DATA
C       USE PDQ FORTRAN CLC2 PROCESSOR
C       USE PDQ FIXED FORMAT SUBROUTINES
C       SENSE SWITCH 2 ON PRINTS INPUT DATA
    8   AN=0.0
        SX=0.0
        SY=0.0
        SXSQ=0.0
        SYSQ=0.0
        SXY=0.0
        READ 10
        PRINT 10
   10   FORMAT(40H
        IF (SENSE SWITCH 2)11,1
   11   PRINT 12
   12   FORMAT(/10X,1HA,10X,1HB/)
    1   READ 2,A,B,M
    2   FORMAT (2F6.0,67X,I1)
        IF(M-9)3,4,4
    3   AN=AN+1.0
        IF(SENSE SWITCH 2)14,16
   14   PRINT 15,A,B
   15   FORMAT(5X,2F10.3)
   16   X=0.43429448*LOGF(A)
        Y=B
        SX=SX+X
        SY=SY+Y
        SXSQ=SXSQ+X*X
        SYSQ=SYSQ+Y*Y
        SXY=SXY+X*Y
        GO TO 1
    4   GMX=EXPF(SX/ (AN*0.43429448))
        AVY=SY/AN
        SDX=SQRTF((SXSQ-SX*SX/AN)/(AN-1.0))
        SDY=SQRTF((SYSQ-SY*SY/AN)/(AN-1.0))
        R=(SXY-SX*SY/AN)/(SQRTF((SXSQ-SX*SX/AN)*(SYSQ-SY*SY/AN)))
        RMA=SDX/SDY
        PRINT 5,AN
    5   FORMAT(// 18HNUMBER OF SAMPLES=,F6.0)
        PRINT 6,GMX,SDX
    6   FORMAT(/14HAVERAGE OF A =,F12.4,10X,10HSTD. DEV.=,F10.6)
        PRINT 9,AVY,SDY
    9   FORMAT (/15HARITH AV OF B =,F12.4,9X,10HSTD. DEV.=,F10.6)
        PRINT 7,R,RMA
    7   FORMAT(//3HR =,F8.4,10X,18HRMA SLOPE (A/B) = ,F8.4)
        PAUSE
        GO TO 8
C       MEANS IN ORIGINAL UNITS, SD S IN LOG UNITS
        END
```

Appendix 4c

```
C       LOG-LOG CORRELATIONS FOR OLEARIA RANI
C       USE PDQ FORTRAN CLC2 PROCESSOR
C       USE  PDQ FIXED FORMAT SUB ROUTINES
        DIMENSION X(19),SA(19),SQA(19),SD(12),SQD(12)
        DIMENSION SPA(12),SPD(12),A(19),D(12),XA(12),XD(12),XP(15),SPP(15)
        DIMENSION AVA(19),GMA(19),SDA(19),AVD(12),GMD(12),SDD(12),YA(12)
        DIMENSION YD(12),YP(15),ZA(12),ZD(12),ZP(15),RMAA(12),RMA[illegible](12)
        DIMENSION RMAP(15),RA(12),RD(12),RP(15)
    1   DO 2 I=1,19
        A(I)=0.0
        SA(I)=0.0
    2   SQA(I)=0.0
        DO 3 I=1,12
        SD(I)=0.0
        SQD(I)=0.0
        SPA(I)=0.0
    3   SPD(I)=0.0
        DO 4 I=1,15
    4   SPP(I)=0.0
        AN=0.0
C       START OF MAIN LOOP
    5   READ 6,X(1),X(2),X(3),X(4),X(5),X(6),X(7),X(8),X(9),X(10),X(11),
       3X(12),X(13),X(14),X(15),X(16),X(17),X(18),X(19),M
    6   FORMAT (19F4.0,3X,I1)
        IF (M-9)8,100,100
    8   AN=AN+1.0
        DO 9 I=1,15
        A(I)=0.43429448*LOG(X(I))
        SA(I)=SA(I)+A(I)
    9   SQA(I)=SQA(I)+A(I)*A(I)
        DO 10 I=16,19
        SA(I)=SA(I)+X(I)
   10   SQA(I)=SQA(I)+X(I)*X(I)
        DO 11 I=1,3
   11   D(I)=0.43429448*LOG(X(I)*X(16))
        DO 12 I=4,6
   12   D(I)=0.43429448*LOG(X(I)*X(17))
        DO 13 I=7,9
   13   D(I)=0.43429448*LOG(X(I)*X(18))
        DO 14 I=10,12
   14   D(I)=0.43429448*LOG(X(I)*X(19))
        DO 15 I=1,12
        SD(I)=SD(I)+D(I)
   15   SQD(I)=SQD(I)+D(I)*D(I)
C       CROSS PRODUCTS,P-S,P-P
        DO 16 I=1,12,3
        XA(I)=A(13)*A(I)
   16   XD(I)=A(13)*D(I)
        DO 17 I=2,12,3
        XA(I)=A(14)*A(I)
   17   XD(I)=A(14)*D(I)
        DO 18 I=3,12,3
        XA(I)=A(15)*A(I)
   18   XD(I)=A(15)*D(I)
        XP(1)=A(1)*A(2)
        XP(2)=A(1)*A(3)
        XP(3)=A(2)*A(3)
        XP(4)=A(4)*A(5)
        XP(5)=A(4)*A(6)
```

Appendix 4c (Continued)

```
      XP(6)=A(5)*A(6)
      XP(7)=A(7)*A(8)
      XP(8)=A(7)*A(9)
      XP(9)=A(8)*A(9)
      XP(10)=A(10)*A(11)
      XP(11)=A(10)*A(12)
      XP(12)=A(11)*A(12)
      XP(13)=A(13)*A(14)
      XP(14)=A(13)*A(15)
      XP(15)=A(14)*A(15)
      DO 19 I=1,12
      SPA(I)=SPA(I)+XA(I)
   19 SPD(I)=SPD(I)+XD(I)
      DO 20 I=1,15
   20 SPP(I)=SPP(I)+XP(I)
      GO TO 5
C     END OF MAIN LOOP
  100 DO 21 I=1,19
      AVA(I)=SA(I)/AN
      GMA(I)=EXP(AVA(I)/0.43429448)
   21 SDA(I)=SQRT((SQA(I)-AN*AVA(I)*AVA(I))/(AN-1.0))
      DO 22 I=1,12
      AVD(I)=SD(I)/AN
      GMD(I)=EXP(AVD(I)/0.43429448)
   22 SDD(I)=SQRT((SQD(I)-AN*AVD(I)*AVD(I))/(AN-1.0))
      DO 23 I=1,12,3
      YA(I)=AVA(13)*AVA(I)
      YD(I)=AVA(13)*AVD(I)
      ZA(I)=(SQA(13)-AN*AVA(13)*AVA(13))*(SQA(I)-AN*AVA(I)*AVA(I))
      ZD(I)=(SQA(13)-AN*AVA(13)*AVA(13))*(SQD(I)-AN*AVD(I)*AVD(I))
      RMAA(I)=SDA(13)/SDA(I)
   23 RMAD(I)=SDA(13)/SDD(I)
      DO 24 I=2,12,3
      YA(I)=AVA(14)*AVA(I)
      YD(I)=AVA(14)*AVD(I)
      ZA(I)=(SQA(14)-AN*AVA(14)*AVA(14))*(SQA(I)-AN*AVA(I)*AVA(I))
      ZD(I)=(SQA(14)-AN*AVA(14)*AVA(14))*(SQD(I)-AN*AVD(I)*AVD(I))
      RMAA(I)=SDA(14)/SDA(I)
   24  RMAD(I)=SDA(14)/SDD(I)
      DO 25 I=3,12,3
      YA(I)=AVA(15)*AVA(I)
      YD(I)=AVA(15)*AVD(I)
      ZA(I)=(SQA(15)-AN*AVA(15)*AVA(15))*(SQA(I)-AN*AVA(I)*AVA(I))
      ZD(I)=(SQA(15)-AN*AVA(15)*AVA(15))*(SQD(I)-AN*AVD(I)*AVD(I))
      RMAA(I)=SDA(15)/SDA(I)
   25 RMAD(I)=SDA(15)/SDD(I)
      YP(1)=AVA(1)*AVA(2)
      YP(2)=AVA(1)*AVA(3)
      YP(3)=AVA(2)*AVA(3)
      YP(4)=AVA(4)*AVA(5)
      YP(5)=AVA(4)*AVA(6)
      YP(6)=AVA(5)*AVA(6)
      YP(7)=AVA(7)*AVA(8)
      YP(8)=AVA(7)*AVA(9)
      YP(9)=AVA(8)*AVA(9)
      YP(10)=AVA(10)*AVA(11)
      YP(11)=AVA(10)*AVA(12)
      YP(12)=AVA(11)*AVA(12)
```

Appendix 4c (Continued)

```
      YP(13)=AVA(13)*AVA(14)
      YP(14)=AVA(13)*AVA(15)
      YP(15)=AVA(14)*AVA(15)
      ZP(1)=(SQA(1)-AN*AVA(1)*AVA(1))*(SQA(2)-AN*AVA(2)*AVA(2))
      ZP(2)=(SQA(1)-AN*AVA(1)*AVA(1))*(SQA(3)-AN*AVA(3)*AVA(3))
      ZP(3)=(SQA(2)-AN*AVA(2)*AVA(2))*(SQA(3)-AN*AVA(3)*AVA(3))
      ZP(4)=(SQA(4)-AN*AVA(4)*AVA(4))*(SQA(5)-AN*AVA(5)*AVA(5))
      ZP(5)=(SQA(4)-AN*AVA(4)*AVA(4))*(SQA(6)-AN*AVA(6)*AVA(6))
      ZP(6)=(SQA(5)-AN*AVA(5)*AVA(5))*(SQA(6)-AN*AVA(6)*AVA(6))
      ZP(7)=(SQA(7)-AN*AVA(7)*AVA(7))*(SQA(8)-AN*AVA(8)*AVA(8))
      ZP(8)=(SQA(7)-AN*AVA(7)*AVA(7))*(SQA(9)-AN*AVA(9)*AVA(9))
      ZP(9)=(SQA(8)-AN*AVA(8)*AVA(8))*(SQA(9)-AN*AVA(9)*AVA(9))
      ZP(10)=(SQA(10)-AN*AVA(10)*AVA(10))*(SQA(11)-AN*AVA(11)*AVA(11))
      ZP(11)=(SQA(10)-AN*AVA(10)*AVA(10))*(SQA(12)-AN*AVA(12)*AVA(12))
      ZP(12)=(SQA(11)-AN*AVA(11)*AVA(11))*(SQA(12)-AN*AVA(12)*AVA(12))
      ZP(13)=(SQA(13)-AN*AVA(13)*AVA(13))*(SQA(14)-AN*AVA(14)*AVA(14))
      ZP(14)=(SQA(13)-AN*AVA(13)*AVA(13))*(SQA(15)-AN*AVA(15)*AVA(15))
      ZP(15)=(SQA(14)-AN*AVA(14)*AVA(14))*(SQA(15)-AN*AVA(15)*AVA(15))
      RMAP(1)=SDA(1)/SDA(2)
      RMAP(2)=SDA(1)/SDA(3)
      RMAP(3)=SDA(2)/SDA(3)
      RMAP(4)=SDA(4)/SDA(5)
      RMAP(5)=SDA(4)/SDA(6)
      RMAP(6)=SDA(5)/SDA(6)
      RMAP(7)=SDA(7)/SDA(8)
      RMAP(8)=SDA(7)/SDA(9)
      RMAP(9)=SDA(8)/SDA(9)
      RMAP(10)=SDA(10)/SDA(11)
      RMAP(11)=SDA(10)/SDA(12)
      RMAP(12)=SDA(11)/SDA(12)
      RMAP(13)=SDA(13)/SDA(14)
      RMAP(14)=SDA(13)/SDA(15)
      RMAP(15)=SDA(14)/SDA(15)
      DO 26 I=1,12
      RA(I)=(SPA(I)-AN*YA(I))/SQRT(ZA(I))
   26 RD(I)=(SPD(I)-AN*YD(I))/SQRT(ZD(I))
      DO 27 I=1,15
   27 RP(I)=(SPP(I)-AN*YP(I))/SQRT(ZP(I))
C     END OF CALCULATIONS
      PRINT 28,AN
   28 FORMAT (/18HNUMBER OF SAMPLES=,F6.0/)
      PRINT 29
   29 FORMAT (32HMEAN LEAF ASH CONTENTS. MO,CU,ZN/)
      PRINT 30,GMA(1),SDA(1),GMA(2),SDA(2),GMA(3),SDA(3)
   30 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 31
   31 FORMAT (/36HMEAN OLD WOOD ASH CONTENTS. MO,CU,ZN/)
      PRINT 32,GMA(4),SDA(4),GMA(5),SDA(5),GMA(6),SDA(6)
   32 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 33
   33 FORMAT (/36HMEAN NEW WOOD ASH CONTENTS. MO,CU,ZN/)
      PRINT 34,GMA(7),SDA(7),GMA(8),SDA(8),GMA(9),SDA(9)
   34 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 35
   35 FORMAT (/34HMEAN FLOWER ASH CONTENTS. MO,CU,ZN/)
      PRINT 36,GMA(10),SDA(10),GMA(11),SDA(11),GMA(12),SDA(12)
   36 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 37
```

Appendix 4c (Continued)

```
   37 FORMAT (/30HASH CONTENTS. ARITHMETIC MEANS)
      PRINT 38
   38 FORMAT (34HLEAVES,  OLD WOOD,NEW WOOD,FLOWERS)
      PRINT 39,AVA(16),AVA(17),AVA(18),AVA(19)
   39 FORMAT (4F9.5)
      PRINT 40
   40 FORMAT (28HMEAN SOIL CONTENTS, MO,CU,ZN)
      PRINT 41,GMA(13),SDA(13),GMA(14),SDA(14),GMA(15),SDA(15)
   41 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 42
   42 FORMAT(/32HMEAN DRY LEAF CONTENTS. MO,CU,ZN)
      PRINT 43,GMD(1),SDD(1),GMD(2),SDD(2),GMD(3),SDD(3)
   43 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 44
   44 FORMAT (/36HMEAN DRY,OLD WOOD CONTENTS. MO,CU,ZN)
      PRINT 45,GMD(4),SDD(4),GMD(5),SDD(5),GMD(6),SDD(6)
   45 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 46
   46 FORMAT (/36HMEAN DRY NEW WOOD CONTENTS. MO,CU,ZN)
      PRINT 47,GMD(7),SDD(7),GMD(8),SDD(8),GMD(9),SDD(9)
   47 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 48
   48 FORMAT (/36HMEAN DRY FLOWER CONTENTS. MO,CU,ZN. )
      PRINT 50,GMD(10),SDD(10),GMD(11),SDD(11),GMD(12),SDD(12)
   50 FORMAT (2F8.2,3X,2F8.2,3X,2F8.2)
      PRINT 49
   49 FORMAT (/42HCORRELATION COEFFICIENTS AND R.M.A. SLOPES)
      PRINT 51
   51 FORMAT (/40HASH WITH SOIL           DRY WT. WITH SOIL)
      DO 53 I=1,12
   53 PRINT 54,RA(I),RMAA(I),RD(I),RMAD(I)
   54 FORMAT (2F7.4,10X,2F7.4)
      PRINT 52
   52 FORMAT (41HINTERELEMENT RELATIONS. MO-CU,MO-ZN,CU-ZN)
      PRINT 55
   55 FORMAT (6HLEAVES)
      DO 57 I=1,3
   57 PRINT 56,RP(I),RMAP(I)
   56 FORMAT (2F7.4)
      PRINT 58
   58 FORMAT (8HOLD WOOD)
      DO 59 I=4,6
   59 PRINT 56,RP(I),RMAP(I)
      PRINT 60
   60 FORMAT (8HNEW WOOD)
      DO 61 I=7,9
   61 PRINT 56,RP(I),RMAP(I)
      PRINT 62
   62 FORMAT (7HFLOWERS)
      DO 63 I=10,12
   63 PRINT 56,RP(I),RMAP(I)
      PRINT 64
   64 FORMAT (4HSOIL)
      DO 65 I=13,15
   65 PRINT 56,RP(I),RMAP(I)
C     GMS IN PPM,ASH AVS IN PER CENT,STD,DEVS IN LOG 10 UNITS
      END
```

Appendix 4d

```
C       CORRELATION CALCULATIONS FOR TRACE ELEMENTS
C       IN SERPENTINE PLANTS (LOG-LOG)
C       USE PDQ FORTRAN CLC2 PROCESSOR
C       USE PDQ FIXED FORMAT SUBROUTINES
C       SENSE SWITCH 2  ON PRINTS INPUT DATA
        DIMENSION A(8),S(8),SQ(8),AV(8),GM(8),SD(8),B(8)
        DIMENSION SP(10),X(10),Y(10),Z(10),R(10),RMA(10)
  101   DO 1 I=1,8
        S(I)=0
        SQ(I)=0
    1   AV(I)=0
        DO 17 J=1,10
   17   SP(J)=0
        AN=0.0
        IF(SENSE SWITCH 9 )102,70
   70   READ 13
        PRINT 13
   13   FORMAT (///10X,30H
        IF (SENSE SWITCH 2)10,12
   10   PRINT 11
   11   FORMAT(//14X,3HCR.,17X,3HNI.,17X,3HCU.,17X,3HCO./)
        PRINT 14
   14   FORMAT( 5X,5HPLANT,6X,4HSOIL,5X,5HPLANT,6X,4HSOIL,5X,5HPLANT,6X,
       14HSOIL,5X,5HPLANT,6X,4HSOIL)
C       START OF MAIN LOOP
   12   READ 3,A(1),A(2),A(3),A(4),A(5),A(6),A(7),A(8),M
    3   FORMAT(8F6.0,31X,I1)
        IF(M-9)4,100,100
    4   AN=AN+1.0
        IF(SENSE SWITCH 2)5,15
    5   PRINT 6,A(1),A(2),A(3),A(4),A(5),A(6),A(7),A(8)
    6   FORMAT(//5X,8F 9.0)
   15   DO 16 I=1,8
        B(I)=0.43429448*LOGF(A(I))
        S(I)=S(I)+B(I)
   16   SQ(I)=SQ(I)+B(I)*B(I)
        X(1)=B(1)*B(2)
        X(2)=B(3)*B(4)
        X(3)=B(5)*B(6)
        X(4)=B(7)*B(8)
        X(5)=B(1)*B(3)
        X(6)=B(1)*B(5)
        X(7)=B(1)*B(7)
        X(8)=B(3)*B(5)
        X(9)=B(3)*B(7)
        X(10)=B(5)*B(7)
        DO 18 J=1,10
   18   SP(J)=SP(J)+X(J)
        GO TO 12
C       END OF MAIN LOOP
  100   DO 19 I=1,8
        AV(I)=S(I)/AN
        GM(I)=EXPF(AV(I)/0.43429448)
   19   SD(I)=SQRTF((SQ(I)-AN*AV(I)*AV(I))/(AN-1.0))
        Y(1)=AV(1)*AV(2)
        Y(2)=AV(3)*AV(4)
```

Appendix 4d (Continued)

```
      Y(3)=AV(5)*AV(6)
      Y(4)=AV(7)*AV(8)
      Y(5)=AV(1)*AV(3)
      Y(6)=AV(1)*AV(5)
      Y(7)=AV(1)*AV(7)
      Y(8)=AV(3)*AV(5)
      Y(9)=AV(3)*AV(7)
      Y(10)=AV(5)*AV(7)
      Z(1)=(SQ(1)-AN*AV(1)*AV(1))*(SQ(2)-AN*AV(2)*AV(2))
      Z(2)=(SQ(3)-AN*AV(3)*AV(3))*(SQ(4)-AN*AV(4)*AV(4))
      Z(3)=(SQ(5)-AN*AV(5)*AV(5))*(SQ(6)-AN*AV(6)*AV(6))
      Z(4)=(SQ(7)-AN*AV(7)*AV(7))*(SQ(8)-AN*AV(8)*AV(8))
      Z(5)=(SQ(1)-AN*AV(1)*AV(1))*(SQ(3)-AN*AV(3)*AV(3))
      Z(6)=(SQ(1)-AN*AV(1)*AV(1))*(SQ(5)-AN*AV(5)*AV(5))
      Z(7)=(SQ(1)-AN*AV(1)*AV(1))*(SQ(7)-AN*AV(7)*AV(7))
      Z(8)=(SQ(3)-AN*AV(3)*AV(3))*(SQ(5)-AN*AV(5)*AV(5))
      Z(9)=(SQ(3)-AN*AV(3)*AV(3))*(SQ(7)-AN*AV(7)*AV(7))
      Z(10)=(SQ(5)-AN*AV(5)*AV(5))*(SQ(7)-AN*AV(7)*AV(7))
      RMA(1)=SD(1)/SD(2)
      RMA(2)=SD(3)/SD(4)
      RMA(3)=SD(5)/SD(6)
      RMA(4)=SD(7)/SD(8)
      RMA(5)=SD(1)/SD(3)
      RMA(6)=SD(1)/SD(5)
      RMA(7)=SD(1)/SD(7)
      RMA(8)=SD(3)/SD(5)
      RMA(9)=SD(3)/SD(7)
      RMA(10)=SD(5)/SD(7)
      DO 20 J=1,10
   20 R(J)=(SP(J)-AN*Y(J))/SQRTF(Z(J))
C     END OF CALCULATIONS
      TYPE 40
   40 FORMAT(5HMEANS)
      DO 41I=1,8
   41 TYPE 42,GM(I)
   42 FORMAT(8F9.0)
      TYPE 43
   43 FORMAT(10HSTD. DEVS.)
      DO 44 I=1,8
   44 TYPE45,SD(I)
   45 FORMAT (8F9.5)
      PRINT 23,AN
   23 FORMAT(/18HNUMBER OF SAMPLES=,F6.0/)
      TYPE 35
   35 FORMAT(24HCORRELATION COEFFICIENTS)
      DO 30J=1,10
   30 TYPE 36,R(J)
   36 FORMAT(10F8.4)
      TYPE 37
   37 FORMAT(17HSLOPES OF R.M.A.S)
      DO 31J=1,10
   31 TYPE 38,RMA (J)
   38 FORMAT (10F8.3)
  102 PAUSE
      GO TO 101
C     GEOMETRIC MEANS IN PARTS PER MILLION
C     STANDARD DEVIATIONS IN UNITS OF LOGARITHM TO BASE TEN
C     SLOPES OF RMAS MAY BE RECIPROCAL OF THAT IN GRAPHS EG NOS 9 AND 10
      END
```

Appendix 4e

```
C       LOG-LOG CORRELATIONS WITH ZN, CA, MG IN SERPENTINE PLANTS
C       USE PDQ FORTRAN CLC2 PROCESSOR
C       USE PDQ FIXED FORMAT  SUBROUTINES
C       SENSE SWITCH 2 ON PRINTS INPUT DATA
        DIMENSION S(8),SQ(8),SP(20),A(8),B(8),X(20),AV(8),GM(8),SD(8)
        DIMENSION Y(20),Z(20),RMA(20),R(20)
      1 DO 2 I=1,8
        S(I)=0.0
      2 SQ(I)=0.0
        DO 3 I=1,20
      3 SP(I)=0.0
        AN=0.0
        READ 4
        PRINT 4
      4 FORMAT(40H
        IF(SENSE SWITCH 2)5,7
      5 PRINT 6
      6 FORMAT(//5X,2HCR,8X,2HNI,8X,2HCU,8X,2HCO,8X,2HZN,8X,2HCA,8X,2HMG,
       36X,5HCA/MG/)
C       START OF MAIN LOOP
      7 READ 8,A(1),A(2),A(3),A(4),A(5),A(6),A(7),M
      8 FORMAT(F6.0,6X,F6.0,6X,F6.0,6X,F6.0,6X,3F6.0,13X,I1)
        IF(M-9)9,100,100
      9 AN=AN+1.0
        A(8)=A(6)/A(7)
        IF(SENSE SWITCH 2)10,12
     10 PRINT 11,A(1),A(2),A(3),A(4),A(5),A(6),A(7),A(8)
     11 FORMAT (8F10.3)
     12 DO 13 I=1,8
        B(I)=0.43429448*LOG(A(I))
        S(I)=S(I)+B(I)
     13 SQ(I)=SQ(I)+B(I)*B(I)
        DO 14 I=1,4
     14 X(I)=B(1)*B(I+4)
        DO 15 I=5,8
     15 X(I)=B(2)*B(I)
        DO 16 I=9,12
     16 X(I)=B(3)*B(I-4)
        DO 17 I=13,16
     17 X(I)=B(4)*B(I-8)
        DO 18 I=17,19
     18 X(I)=B(5)*B(I-11)
        X(20)=B(6)*B(7)
        DO 19 I=1,20
     19 SP(I)=SP(I)+X(I)
        GO TO 7
C       END OF MAIN LOOP
    100 DO 20 I=1,8
        AV(I)=S(I)/AN
        GM(I)=EXP(AV(I)/0.43429448)
     20 SD(I)=SQRT((SQ(I)-AN*AV(I)*AV(I))/(AN-1.0))
        DO 21 I=1,4
        Y(I)=AV(1)*AV(I+4)
        Z(I)=(SQ(1)-AN*AV(1)*AV(1))*(SQ(I+4)-AN*AV(I+4)*AV(I+4))
```

Appendix 4e (Continued)

```
   21 RMA(I)=SD(1)/SD(I+4)
      DO 22 I=5,8
      Y(I)=AV(2)*AV(I)
      Z(I)=(SQ(2)-AN*AV(2)*AV(2))*(SQ(I)-AN*AV(I)*AV(I))
   22 RMA(I)=SD(2)/SD(I)
      DO 23 I=9,12
      Y(I)=AV(3)*AV(I-4)
      Z(I)=(SQ(3)-AN*AV(3)*AV(3))*(SQ(I-4)-AN*AV(I-4)*AV(I-4))
   23 RMA(I)=SD(3)/SD(I-4)
      DO 24 I=13,16
      Y(I)=AV(4)*AV(I-8)
      Z(I)=(SQ(4)-AN*AV(4)*AV(4))*(SQ(I-8)-AN*AV(I-8)*AV(I-8))
   24 RMA(I)=SD(4)/SD(I-8)
      DO 25 I=17,19
      Y(I)=AV(5)*AV(I-11)
      Z(I)=(SQ(5)-AN*AV(5)*AV(5))*(SQ(I-11)-AN*AV(I-11)*AV(I-11))
   25 RMA(I)=SD(5)/SD(I-11)
      Y(20)=AV(6)*AV(7)
      Z(20)=(SQ(6)-AN*AV(6)*AV(6))*(SQ(7)-AN*AV(7)*AV(7))
      RMA(20)=SD(6)/SD(7)
      DO 26 I=1,20
   26 R(I)=(SP(I)-AN*Y(I))/SQRT(Z(I))
C     END OF CALCULATIONS
      PRINT 27
   27 FORMAT (//20HMEANS AND STD. DEVS./)
      DO 29 I=1,8
   29 PRINT 28,GM(I),SD(I)
   28 FORMAT(F10.4,2X,F10.6)
      PRINT 30,AN
   30 FORMAT(//18HNUMBER OF SAMPLES=,F6.0/)
      PRINT 31
   31 FORMAT (37HCORRELATION COEFFICIENTS,R.M.A. SLOPE/)
      DO 32 I=1,20
   32 PRINT 33,R(I),RMA(I)
   33 FORMAT(10X,F10.6,10X,F8.3)
      PAUSE
      GO TO 1
C     CA AND MG IN  PERCENT,OTHER ELEMENTS IN PPM
C     STD DEVS IN LOG UNITS
      END
```

Appendix 5.

Publications Arising from This Thesis.

1. Brooks, R.R. and Lyon, G.L. (1966). Biogeochemical prospecting for molybdenum in New Zealand. N.Z. Jl. Sci. 9, 706-718.

2. Lyon, G.L. and Brooks, R.R. (1969). The trace element content of Olearia rani and its application to biogeochemical prospecting. N.Z. Jl. Sci. 12, 200-206.

3. Lyon, G.L., Brooks, R.R., Peterson, P.J. and Butler, G.W. (1968). Trace elements in a New Zealand serpentine flora. Plant and Soil 29, 225-40.

4. Lyon, G.L., Peterson, P.J. and Brooks, R.R. Chromium-51 distribution in tissues and extracts of Leptospermum scoparium J.R. et G. Forst. (Myrtaceae). Submitted to Planta.

5. Lyon, G.L., Peterson, P.J. and Brooks, R.R. Chromium-51 transport in the xylem sap of Leptospermum scoparium J.R. et G. Forst. (Myrtaceae). N.Z. Jl. Sci. (in press).