BIOGEOCHEMICAL STUDIES
OF
NICKEL AND COPPER
IN
NEW ZEALAND

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ABSTRACT

The accumulations of various metals by some indigenous trees growing on the Riwaka Basic Complex, North-west Nelson, New Zealand, were investigated by the application of statistical techniques to biogeochemical data. Particular reference was given to nickel and copper to evaluate the usefulness of plant analysis as a prospecting tool.

Preliminary investigations showed that serious errors could result from the methods of sampling plants and soils and sampling procedures were adopted to minimise these errors. In addition, errors arising from atomic absorption analysis were found to be significant for some metals.

Leaves and twigs from three *Nothofagus* species, *N.racemosa* and *Q.acutifolia* as well as their associated soils, were collected and analysed for nickel, cobalt, copper, zinc, chromium, calcium, magnesium, manganese and potassium. The plants were also analysed for iron. These results showed that each species accumulated different, but related, amounts of various metals and that they distributed these metals in different ways between their leaves and twigs.

N.truncata and *N.fusca* which are closely related genetically, accumulated metals to similar degrees, while *N.menziesii* which is not closely related to the other *Nothofagus* species accumulated metals to differing degrees.

Relationships between the metal concentrations in the plants and in the soils were evaluated by computing correlation coefficients. The best correlations for nickel were obtained for the *Nothofagus* genus although the other species also showed highly significant correlations. The *Nothofagus* genus also showed the best correlation for copper.

In view of the above results, a more extensive study of the *Nothofagus* genus was carried out. A second survey was undertaken in the same area in which leaf samples of this genus as well as their associated soils were collected. While the metal concentrations in the soils collected in this survey compared well to those collected previously, the metal concentrations in the plants, in general, did not show good agreement.

Trend analysis was used to compare in detail the nickel and copper contents in the leaves of the *Nothofagus* genus with the concentrations of these metals in the soils. It was shown by comparison of the trend surfaces and residuals that the accumulation of nickel was determined primarily by the concentration of nickel in the soil, whereas for copper the accumulation by the plant was a function primarily of the specific requirement of the plant for this metal.

Multiple regression analysis was used to improve the prediction of the copper and nickel concentrations in the soil from the concentrations of these metals in the leaves of the *Nothofagus* species, by making quantitative allowance for the processes influencing the

accumulation of these metals by the plants. Improvements of between 25% and 35% were obtained at the 90% confidence level.

Inter-metal ratios in the leaves were considered as possible indicators of nickel and copper concentrations in the soil but the results were discouraging.

Studies were made of the locations and chemical forms of nickel, copper, zinc and iron in both freeze-dried and fresh leaves from some trees growing on the Complex. Atomic absorption spectrophotometry was used to measure the concentrations of these metals in both plant extracts and on the electrophoresis and chromatography papers used to separate the metal complexes in the extracts. Results indicated that the major part of the nickel present in the leaves was not contained in cell organelles nor was it bound to cell walls, but existed as a positively charged complex in either the cytoplasm and/or the vacuole. Copper, zinc and iron were distributed differently with varying fractions, depending on the metal, existing predominately as anionic complexes.

It was concluded that the research embodied in this thesis had illustrated the application of statistical techniques to biogeochemical studies, showed that biogeochemical prospecting for nickel in New Zealand was feasible and that methods of total analysis for metals could be applied to the study of microgram amounts of metals in biological systems.

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GENERAL INTRODUCTION

Life, in all its many forms, owes its existence on this planet, in large part, to the natural cycling of metals in the biosphere.

In recent years, man's concern for this cycle has been increased, not only by the extent to which he is disturbing the natural distributions of elements but also by the realisation that the number of elements known to be essential to the metabolic functions of his body, is increasing more rapidly than his knowledge of the way these metals behave in his environment.

Mertz (1970) noted that there are now twentytwo elements known to be essential to human nutrition and Allaway (1968) has pointed out the exceedingly complex system involved in the environmental cycling of trace elements (Figure I - 1).

Although our knowledge of this system as a whole is very meagre, there are some parts of it which have attracted considerable attention in recent years, in particular the rock / soil / plant segment.

It is a generally accepted fact that the continued existence of civilization in its present form depends to a large extent on a continuous and ever-increasing supply of raw materials. In particular, its needs for minerals, be they fossil fuels for energy or non-metallic or metallic elements, has grown very rapidly during the last century.

The vital importance of new sources of metals to our material well-being has initiated a vast field of research into new techniques which can either pinpoint ore deposits in a large

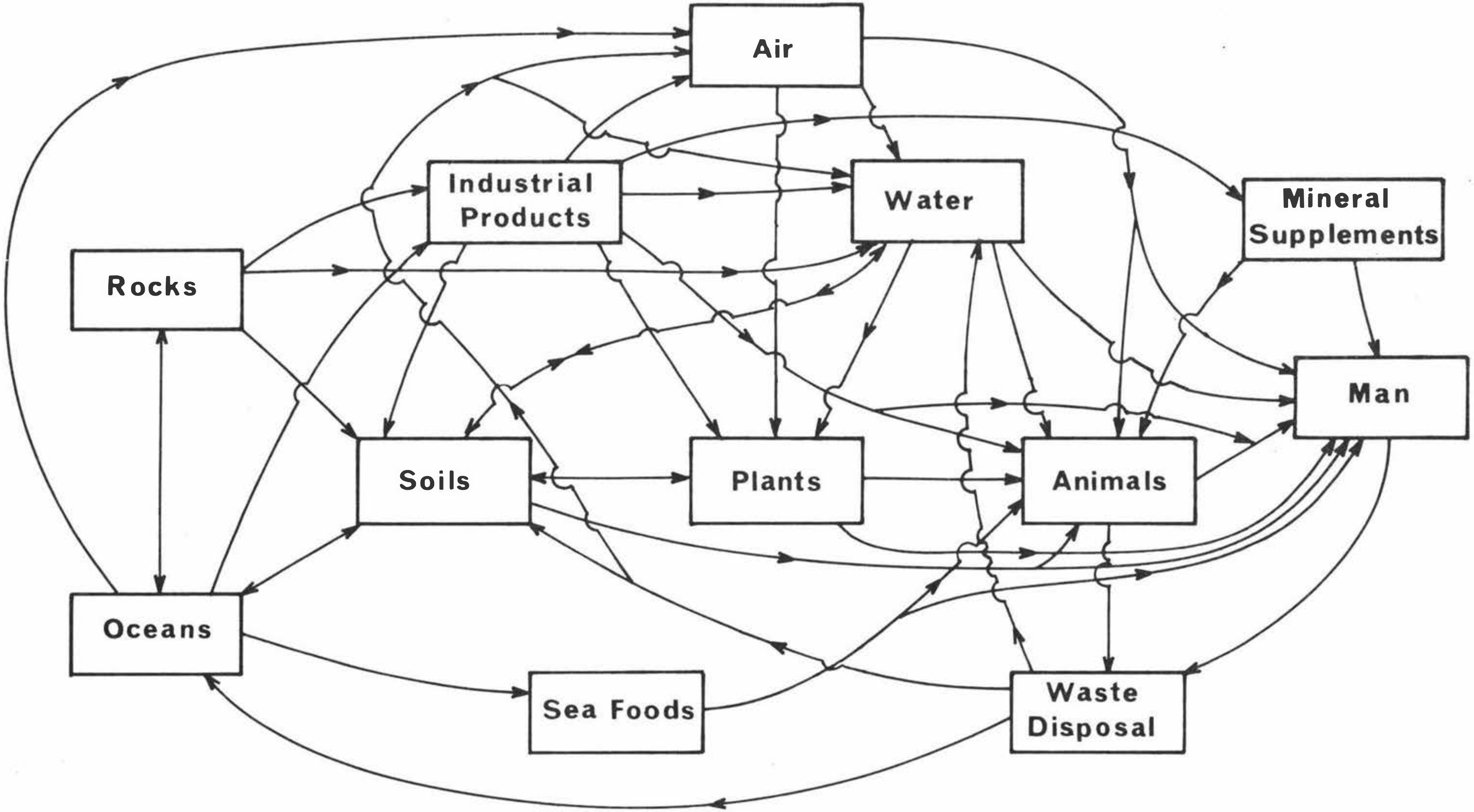


Figure I-1. Pathways of environmental movement of trace elements. (after Allaway, 1968) area or can detect mineralisation under an overburden. It is in the development of these techniques that the geochemist has turned to the natural trace element cycle for assistance and in this respect his observations are of direct significance to our understanding of trace element cycling in the environment.

The findings of the scientist studying aspects of mineral nutrition can assist the geochemist in his search for mineral deposits and the vast volume of knowledge accumulated by the geochemist can provide the scientist with valuable information relating to the movement of trace elements in the biosphere. The study of geochemical prospecting methods and the study of the environmental cycling of trace elements, particularly metals, therefore have a common foundation and the results of one are of general relevance to the other.

As already mentioned, the goals of the geochemist are to locate ore bodies in a large area or under an overburden.

In the former case, the analysis of stream sediments for trace metals is the most popular method developed to date. It is assumed that the sediment represents a composite sample of the soil and unweathered rock in the area drained by the stream. A mineralised zone in the catchment area will be indicated by anomalous concentrations of the ore metal in the stream sediment. This method has been successfully used over very large areas. (Nichol et al., 1969; Garrett and Nichol, 1967.)

Apart from the many geophysical methods such as self potential, induced polarisation and electromagnetics, the detection of ore bodies at depth is generally attempted by analysing small samples of soils taken over the area thought to contain the deposit. Under suitable weathering and topographical conditions, the ore metal will be dispersed either chemically or mechanically into the upper regions of the overlying soil. This secondary dispersion halo, as it is usually called, may be considerably larger than the actual ore body, thus forming a larger "target" area in the soil than in the host rock. The larger the dimensions of this halo, the easier the problems become of locating it. These geochemical prospecting techniques have been discussed in detail by Hawkes and Webb (1962).

Although soils and stream sediments are the most common parts of the trace element cycle being utilized for geochemical prospecting at present, there is a larger segment of the cycle of potential use for detecting ore bodies. This segment contains the rock / soil / plant system. Methods of prospecting based on this system have been used with success in the past and are again being actively considered by mining companies.

These methods are geobotanical prospecting and its associated technique, biogeochemical prospecting.

The first of these, geobotanical prospecting, depends on the association of unusual plant communities with soils containing high concentrations of metals. Visual observation of either the community as a whole, or the presence or absence of a particular plant species, can in favourable circumstances, delineate zones of mineralisation.

Investigations of the geobotanical method have been very extensive, particularly in Russia where it has been developed to a high degree (Chikishev, 1965; Malyuga, 1964; Viktorov et al., 1964.)

In the U.S.A., the most impressive results have been obtained by Cannon and her co-workers of the U.S. Geological Survey in their studies on the uranium deposits of the Colorado Plateau (Cannon, 1952; 1957; 1959; 1960a; 1960b; Cannon and Starrett, 1956). Many of these deposits contained 100p.p.m. to 1000p.p.m. selenium which gave rise to high concentrations of this metal in the overlying soil. It was found that some species of the Astragalus genus grew preferentially in soils high in selenium and by mapping the distributions of these species, seleniferous soils were located. Since these soils were usually derived from uranium ore bodies, these species were useful as "indicator plants" for uranium. Five ore bodies were discovered solely on the basis of indicator plant data (Cannon, 1960a).

Nicolls et al, (1965) investigated geobotanical methods of prospecting in the Dugald River area of Australia. They found that toxic concentrations of copper and lead in the soil determined the assemblage of plants, but that excessive amounts of zinc in the soil did not appear to influence the plant community. A copper anomaly in the surface soil caused by hitherto unknown copper mineralisation was disclosed by the presence of two species.

One of the most extensive studies of the influence of nickel mineralisation on plant assemblages was made by Wild (1970) in Rhodesia. In a study of 28 areas containing high concentrations of nickel in the soil, he recorded 262 angiosperm species on soils containing more than 2000p.p.m. nickel. Often the vegetation on the nickel anomalies was derived from the associated serpentine vegetation. Wild suggested that a form of Dicoma macrocephala was perhaps the best indicator plant for nickel in Rhodesia but it occurred on only 7 out of the 28 anomalies studied.

The associated technique to geobotanical prospecting, biogeochemical prospecting, relies on the movement of metals from the parent rock, through the soil and into the plant tissues and the analysis of these tissues to indicate anomalous concentrations of metals in the substrate. The development of this technique has largely followed the growth of analytical methods for metals and from the pioneering work of Brundin (1939) in Sweden and Tkalich (1938) in Eastern Siberia, biogeochemical prospecting research has expanded rapidly until today when fully automated analyses and detailed statistical evaluations by computer are commonplace.

The most outstanding factor in favour of plant analysis to detect mineralisation, is the fact that trees with extensive root systems obtain their metal nutrients from a large volume of soil. Therefore, in the absence of any complicating factors, the concentration of a metal in the plant tissue will be a representative value for the total soil sampled by the roots, in contrast to the small soil sample usually collected in a geochemical prospecting survey. Thus, plant analyses would ideally result in less random error than is normally associated with geochemical soil samples.

In addition to the bulk sample of soil from which the plant accumulates metal ions, advantage can be taken of the deep root systems of some species to detect anomalous metal concentrations at depth. This latter capability has been utilized with considerable success by Kleinhampl and Koteff (1960) to detect uranium mineralisation on the Colorado Plateau. These workers recorded that conifer samples effectively indicated ore deposits at depths as great as 40 feet in one area and 70 feet in another. Cannon (1960a) concluded that tree analysis could be used successfully to outline uranium mineralisation to a maximum depth of 70 feet in this same area.

The deep root systems of some plant species can also be useful to indicate anomalous metal concentrations under a considerable depth of overburden. Rankama (1940) in Finland obtained positive indications of nickel mineralisation through ten feet of glacial drift and in Canada, Warren and Delavault (1949) detected zinc anomalies in plants over a lead-zinc deposit when the overburden was at least 30 feet thick.

Keith (1968) by the analysis of the covering vegetation in the Upper Mississippi Valley district, successfully detected lead and zinc mineralisation under an overburden of loess where no soil anomalies were obvious. However, where loess was absent, he found that soil analysis gave better results than plant analysis.

Yet another factor in favour of plant analysis rather than soil analysis to detect mineralisation is the ability of some plants to contain higher concentrations of certain metals in the ash of their leaves and twigs, than in the underlying soil. This results in the plants being the more sensitive prospecting tool. Such a case is described by Carlisle and Cleveland (1958) in their studies of the biogeochemical method for the detection of molybdenum in regions of molybdenum mineralisation.

Another example of the use of the molybdenum concentration in plants is given by Warren and Delavault (1965).

They reported a biogeochemical prospecting survey in which approximately 1,000 tree samples were taken over an area of approximately 700 acres. The twigs of three species of trees, Balsam (Abies lasiocarpa), Pine (Pinus contorta), and Spruce (Picea glauca) were analysed for copper and molybdenum. It was found that although the concentrations of both these metals in the twig ash delineated two areas of ore-bearing potential, the molybdenum anomaly shown by the plant analysis was larger than the copper anomaly.

Other applications of the biogeochemical method have been made with varying degrees of success.

Webb and Millman (1951) in their studies on the Nigerian lead-zinc belt found that the lead, zinc and silver concentrations in the twigs and leaves of savannah trees increased in the proximity of mineralisation and they concluded from their results that the heavy metal content of twigs, could, under favourable circumstances assist in the location of buried ore deposits. Nicolls et al.(1965) also showed that in the Dugald River area of Australia, plant analyses for zinc reflected the soil concentrations of this metal and that biogeochemical prospecting was an effective tool for the detection of zinc mineralisation.

More recently, Warren et al.(1964; 1968) showed that in Canada, the Douglas Fir (Pseudotsuga menziesii) can accumulate from ten to one hundred times more arsenic than any of the trees or lesser plants associated with it. They suggested the possible use of this species to detect arsenic dispersion halos from ore deposits containing minor amounts of this metal. This case illustrates the wider potential of biogeochemical prospecting that may be realised by further investigations.

Unfortunately however, some results have been obtained, for copper in particular, which have not been encouraging to a worker contemplating a biogeochemical survey. Huff (1969) found that the copper concentrations in mesquite plants in Arizona were moderately high even when the soil concentrations were low. He concluded that these plants were not good exploratory guides for this metal. Also Lovering et al. (1950) found that in semi-arid areas, plant analyses were not good indications of copper ore deposits.

In general, it can be said that successful results obtained for a particular metal in one area do not necessarily imply that a survey for this metal in another area using a different plant will also be successful.

Some workers have suggested factors which may influence biogeochemical prospecting results (Carlisle and Cleveland, 1958; Fortescue and Hornbrook, 1967; Shacklette, 1962; Webb and Millman, 1951).

These factors may be broadly classified into three groups:

(a) The particular plant species sampled,

(b) the particular part of the plant sampled, and

(c) the factors which influence the movement of metal ions from the primary source, the bedrock, into the part of the plant taken for analysis.

There is ample evidence to illustrate the necessity of choosing carefully the species and plant part for a particular sampling program, (Shacklette, 1962; Warren *et al.*, 1955), but there has been very little work carried out to understand the various mechanisms in category c.

Fortescue and Hornbrook (1967) presented a scheme for the geochemical cycle (Figure I - 2) in which they classified the system from primary magma through magmatism, crystalline rocks, to soils, as part of the major geochemical cycle while the biosphere was included as part of the minor geochemical cycle. In applying the biogeochemical method to prospecting, it is necessary to understand that part of the geochemical cycle from crystalline rocks to the biosphere. This part of the geochemical cycle contains the components of the environmental trace element cycle discussed by Allaway (1968) and mentioned earlier in this introduction. The segment of this trace element cycle which is of relevance to biogeochemical prospecting contains both geochemical and biochemical processes, and may appropriately be called the "Biogeochemical Cycle." The main components of this cycle are illustrated diagrammatically in Figure I - 3.

A particular metal analysed in some plant tissue will have followed the cycle through the processes of weathering, ion exchange, possible organic chelation, root absorption, translocation and finally storage to some degree in the leaves or twigs. Each of these processes will have influenced the cycling of the metal in different ways and to different extents. The net result of this will be that the concentration of the metal found in the leaf tissue will be a function not only of the concentration in

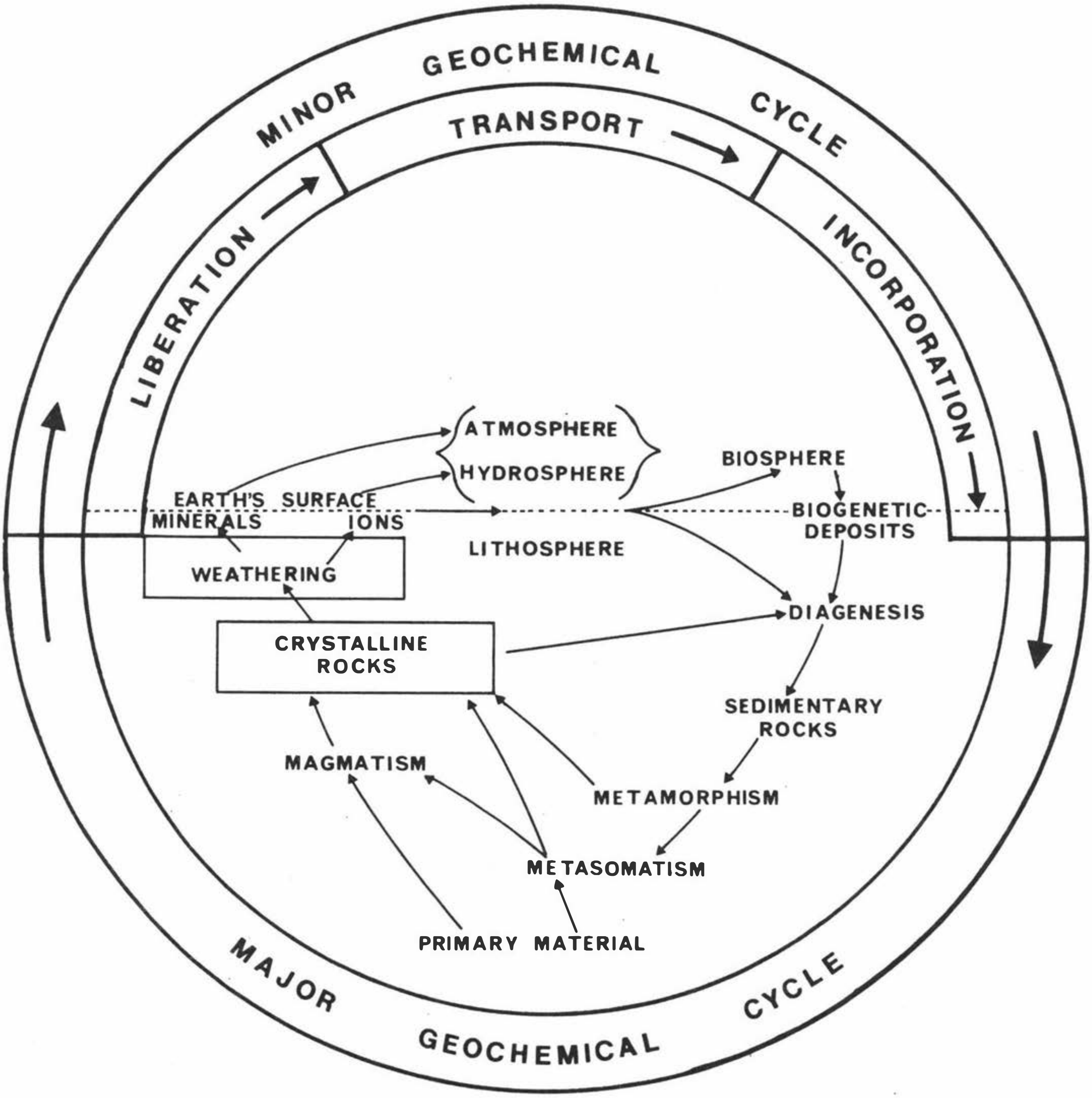


Figure I-2. The Geochemical Cycle (after Fortescue and Hornbrook, 1967)

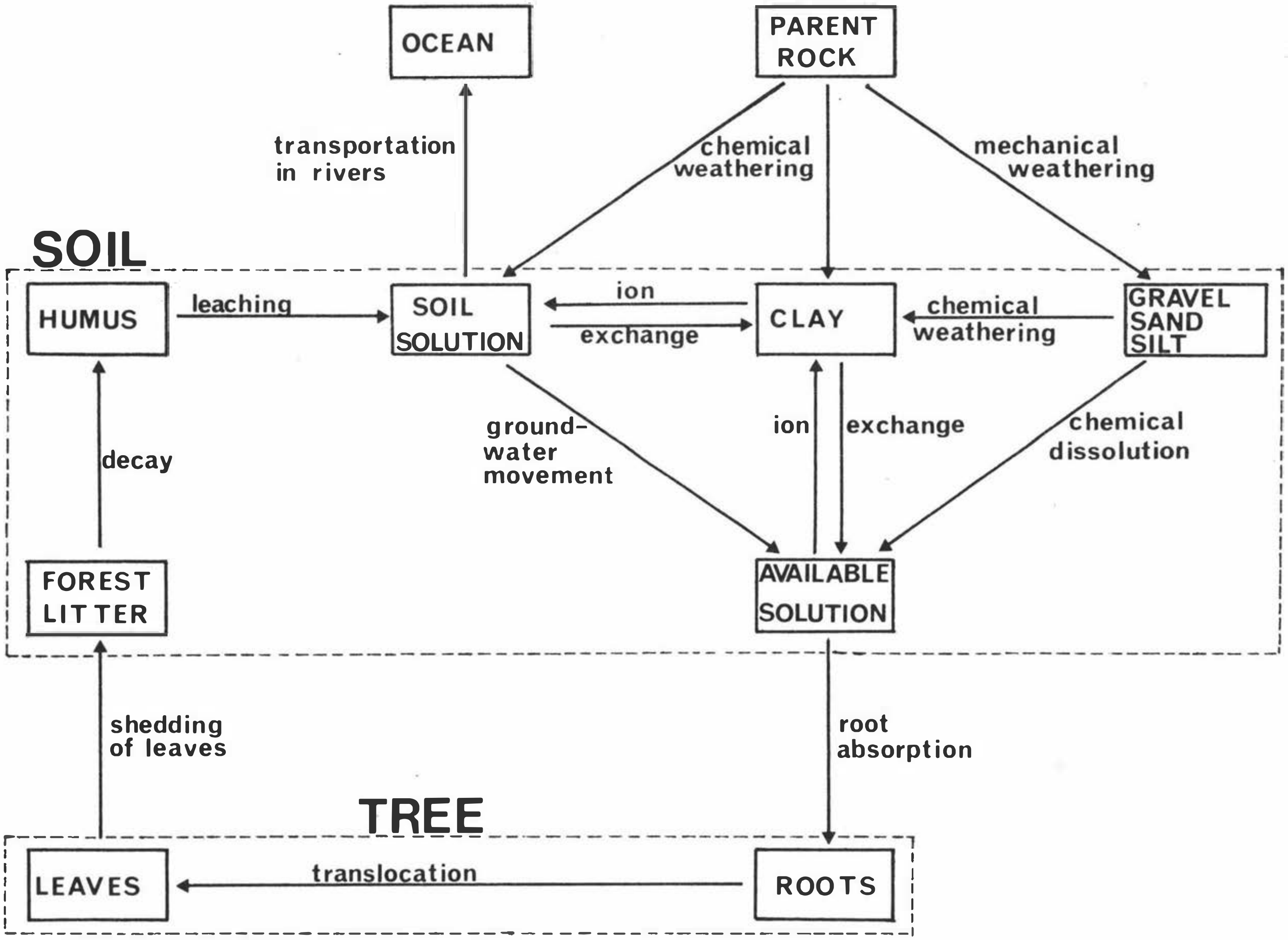


Figure I-3. The Biogeochemical Cycle.

the bedrock, but also of all the processes it has undergone in the movement around the biogeochemical cycle.

There are two different approaches to the problem of understanding the influences these processes have on the trace metal balance between plants and their associated soils.

The first approach is to study each factor in turn, by closely controlling the other factors. This is usually achieved by growing the plants of interest in a culture media under strictly controlled conditions. Where this is impractical, plants and their soils are transferred from their natural environment into the laboratory. This latter method, however, allows only minimal control of the biogeochemical processes. Fortescue and Hornbrook (1967) in their studies of biogeochemical methods at the Geological Survey of Canada grew willow saplings in sterile soil and studied their uptake of nickel, cobalt, lead, silver and strontium. In another series of experiments, young birch trees were transferred, along with their associated soils, from their natural environment in the forest back to the laboratory. Anderson and Kurtz (1954) also used this approach in an attempt to account for the great variability in the accumulation of uranium by plants growing in the field. They grew plants by the nutrient-culture method and considered the factors which influenced the accumulation of uranium.

An alternative approach to the understanding of the processes involved in the biogeochemical cycle is relatively new and has become possible with the advent of large and fast computers. This is the statistical approach. With this technique, no attempt is made to control any of the environmental conditions. Observations are made in the field and these results are subjected to statistical analysis to try and deduce the processes which gave rise to the observed data.

Multivariate analysis has been applied to a variety of geochemical problems (Agterberg, 1967; Middleton, 1963; Nichol and Webb, 1967; Nichol et al., 1969). In particular, factor analysis (Cameron, 1968;1969; Garrett, 1967; Garrett and Nichol, 1969), trend analysis (Allen and Krumbein, 1962; Connor and Miesch, 1964, Nichol et al., 1969) and multiple regression analysis (Rose et al., 1970; Sinclair and Percy, 1969) have been widely used.

The use of multivariate analysis has not, however, been reported for biogeochemical studies and to the author's knowledge this thesis represents the first such application.

In New Zealand, biogeochemical prospecting has been investigated for molybdenum at Copperstain Creek, North-west Nelson (Brooks and Lyon, 1966; Lyon and Brooks, 1969) where it was shown that the molybdenum concentrations in the leaf ash of Olearia rani were useful to indicate anomalous concentrations of this metal in the soil.

Uranium has also received attention and it was shown by Whitehead and Brooks (1969) that the biogeochemical method could be used successfully to prospect for this metal in the Buller Gorge.

Other metals investigated include zinc and lead in the Te Aroha area (Nicolas and Brooks, 1969).

The project described in this thesis was initiated to evaluate the feasibility of using plant analysis in the search for nickel and copper in New Zealand.

No nickel deposits of major economic significance are known in New Zealand but a recent discovery in the Riwaka Basic Complex, North-west Nelson, (Gill and Johnston, 1967) attracted considerable attention.

This Complex, consisting of a belt of basic and ultrabasic rocks extending from south of the Baton River, north to Rameka Creek, has intruded the lower Paleozoic sediments of the Mount Arthur and Onekaka formations (see Figure I - 4). Pyroxenite with minor gabbro is the most extensive rock type of the Complex while peridotite exists as discreet outcrops within the pyroxenite.

The presence of massive copper and nickel sulphides in the Graham River area of the Complex was reported by Gill and Johnston (1967) and it was on the basis of this discovery that McIntyre Mines (N.Z.) Ltd. commenced geochemical prospecting in the area.

It was with the permission and assistance of McIntyre Mines (N.Z.) Ltd. that the project described in this thesis was undertaken on part of their concession area. The particular region of the Complex which was chosen for this project was 8 to 10 miles south of the Graham Valley and was known to contain copper and nickel mineralisation but the grade or quantity present was not known. (see Figure I - 4).

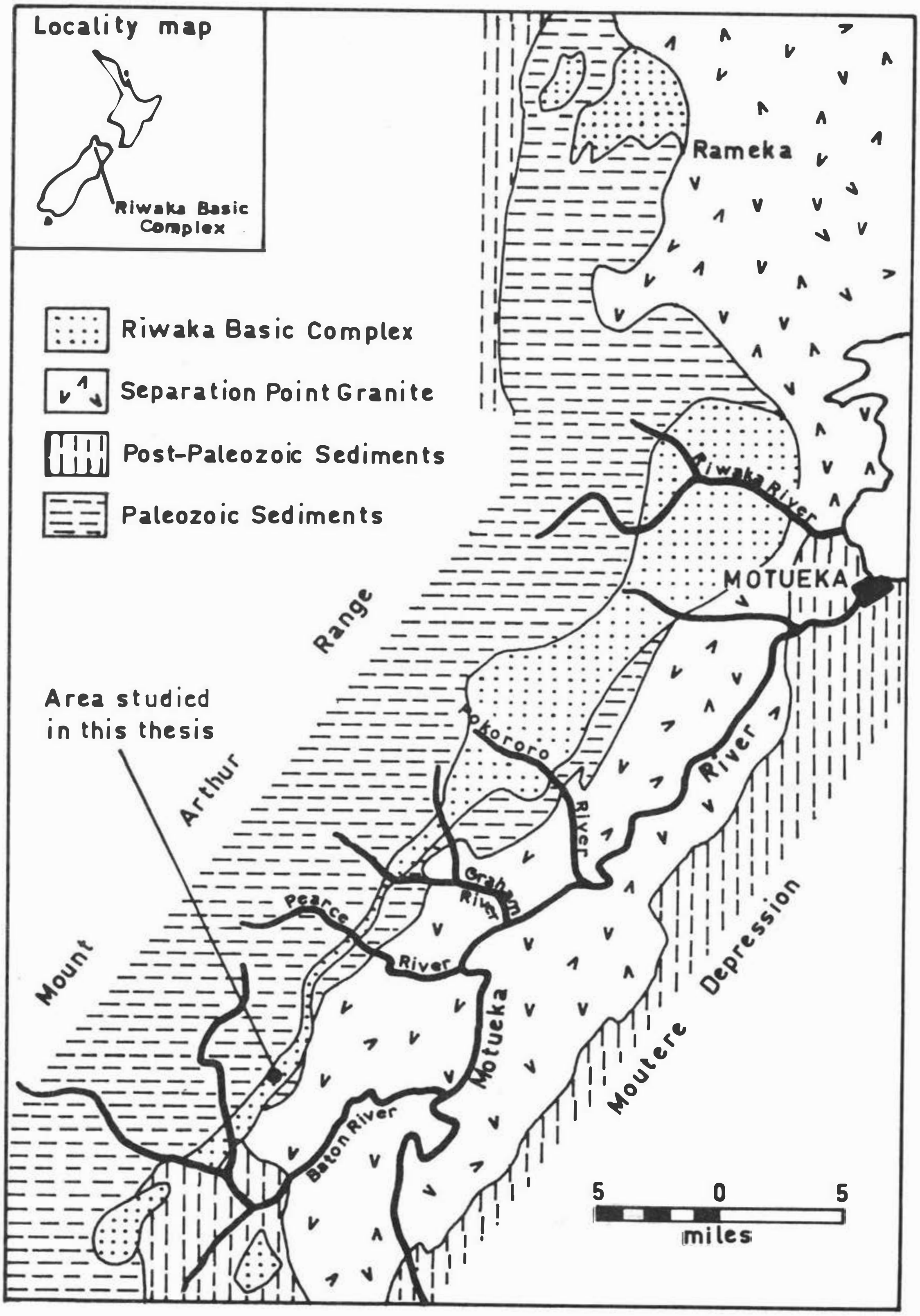


Figure I-4. Geological sketch map of the eastern part of North-west Nelson (after Gill and Johnston, 1967)

The main objectives of this thesis are summarized as follows:

1. To evaluate the errors involved in the analytical and sampling procedures by which the plant and soil data were obtained. This investigation was of vital relevance to the success of the statistical procedures used.

2. To study the concentrations of a number of metals in different plant species and to select a particular species for a more detailed investigation of its metal content.

3. To select an appropriate statistical procedure and to illustrate the way this procedure could be used to gain information about the accumulation of metals by plants.

4. To illustrate a mathematical approach to the prediction of metal concentrations in the soil from the metal concentrations in the plant tissue when the accumulation of the metal by the plant is influenced by unknown factors.

5. To develop computer programmes to implement these statistical techniques on the available computer.

6. To study statistically the influences of high concentrations of nickel and copper in the soil on the metal concentrations and the inter-metal ratios in the plant tissue. The application of these ratios as indicators of copper and nickel mineralisation was also considered.

7. To investigate the use of atomic absorption spectrophotometry in assisting the qualitative and quantative study of microgram amounts of trace metal complexes in plant tissues.

8. To apply the techniques developed in 7. above to a brief study of the chemical forms of trace metals, particularly nickel, in the leaves of some trees on the Riwaka Basic Complex.

SECTION II

ANALYTICAL TECHNIQUES

A. INSTRUMENTS AND CHEMICALS.

All quantitative analyses for metals were carried out by atomic absorption spectrophotometry using an air/acetylene flame except for potassium which was analysed by flamephotometry in an air/coal gas flame. A Techtron Model AA3 was used for atomic absorption spectrophotometry in the early part of the work. Later a Varian-Techtron Model AA5 became available and this was used for the majority of the analyses.

The flame-photometer used was a Gallenkamp Flame Analyser FH500.

Measurement of soil pH was carried out on a Radiometer 28 pH meter.

Aqueous standards were made from Analytical Grade or Spectrographically-pure (Johnson, Matthey & Co. Ltd.) reagents and stored at a concentration of 1000p.p.m. in 2M hydrochloric acid. These were diluted immediately prior to use.

B. SENSITIVITIES AND LIMITS OF MEASUREMENT

Sensitivity in atomic absorption analysis is generally defined as the concentration of a metal in solution necessary to give a signal of 1% absorption.

Values obtained on the AA5 for the metals measured in this thesis are given in Table II - 1. These values were obtained at the operating parameters of flame position and composition which were usually achieved under normal routine analysis conditions. They are not necessarily the maximum sensitivities that could be obtained. Aqueous solutions of 2M hydrochloric acid were used for all metals except calcium, manganese, magnesium and potassium which were determined in a 0.8% strontium nitrate solution (SECTION II - E). All other operating parameters were used as recommended by the manufacturers.

The limit of detection has had a number of definitions (Barney, 1967) but probably the most used is the concentration corresponding to twice the standard deviation of the noise level. A reading at this concentration has an approximately 95% chance of not being due to noise. While this may be suitable for comparative purposes, in practice it can be misleading. It was found during the course of this project that to obtain a reasonable degree of analytical precision (<5.0%) in routine analysis, it was necessary to consider the "limit of measurement" as about four times the noise level. These values are given in Table II - 1. Maximum scale expansion of approximately 10X was used.

Under normal circumstances a measurement could be obtained on 0.3ml, and from the limit of measurement this gave the "lowest amount measureable" with <5.0% precision (Table II - 1).

TABLE II - 1

Details of the analytical capabilities of the methods used.

Metal	Wavelength Å	Sensitivity[‡] p.p.m.	Limit of measurement[+] p.p.m.	Lowest measureable[++] amount x 10^{-9}gm
nickel	2320.0	0.14	0.112	37
cobalt	2407.3	0.17	0.130	43
copper	3247.5	0.10	0.080	26
zinc	2138.6	0.02	0.024	8
chromium	3578.7	0.20	0.160	53
iron	2483.3	0.13	0.104	35
calcium	4226.7	0.29	0.240	80
magnesium	2852.1	0.01	0.008	3
manganese	2794.8	0.11	0.080	33
potassium	-	-	0.200	67

[‡] Concentration to give 1% absorption

[+] Concentration = 4 x noise

[++] Limit of measurement x 0.3ml.

C. TREATMENT OF PLANT SAMPLES.

1. Preliminary Treatment.

On arrival in the laboratory, plant samples were removed from their plastic bag containers and leaves and twigs were dried overnight at 80°C in separate paper bags.

Although soil contamination is a possible source of error in plant analysis (Mitchell, 1960) this was assumed to be of very minor importance in the area surveyed since there was little exposed soil, a dense vegetation canopy and a relatively high rainfall. For these reasons, plants were not washed before drying.

2. Ashing Techniques.

For atomic absorption analysis, it is necessary for the sample to be in solution and for plant samples there are two main ways of achieving this:

(a) dry ashing at 450 to 500°C in a muffle furnace and

(b) wet ashing with mixtures of concentrated perchloric, nitric and sulphuric acids at approximately 100°C. (Scharrer and Munk, 1956).

The choice between these two methods depends on the following factors:

1. If volatile metals such as arsenic, selenium, or mercury are to be determined, it is necessary to use the wet method since these metals are all vapourised to varying extents at 450°C. Other less volatile metals such as lead, zinc and cadmium can also be lost during dry ashing. (Mitchell, 1964.)

2. If low levels of metals are to be determined, then wet ashing has the disadvantage that the concentrated acids must be of exceptional purity to avoid excessive contamination of the sample. In the analysis of metals such as iron and zinc this purity is sometimes difficult to achieve. The usual maximum concentrations quoted for analytical grade concentrated acids are of the order of 1p.p.m. iron and 1p.p.m. heavy metals. If, for example, 20.0ml of concentrated acids was used to ash a sample of 1gm (of vegetation or soil), then the sample could be contaminated to the extent of 20μg of iron and 20μg of heavy metals. It must be said, however, that most analytical-grade acids do not contain these amounts, but even at lower levels the problem is still significant. Acid blank samples can be used to correct for this contamination if the sample contains appreciably more metal than the blank.

Any perchloric, nitric or sulphuric acid remaining in the solution after wet ashing may cause interference during the atomic absorption determinations (Maruta et al., 1970; Govindaraju, 1970).

With dry ashing, on the other hand, the ash is usually dissolved in 2 M hydrochloric acid, which, because of the low amounts of acid present, results in negligible contamination of the sample.

3. Loss of metals during dry ashing.

(a) Methods.

To evaluate the loss of different metals at different temperatures, the following procedure was carried out:

One circle of Whatman (No.541, 7cm) filter paper was added to each of ten test tubes. 10µg each of copper, zinc and nickel were added to each tube and the solutions dried at 30°C. They were then ashed at (a) 450°C and (b) 500°C for 12 hours in two experiments. After cooling, hydrochloric acid (2.0ml) was added to each tube and the solutions analysed. No corrections were necessary for the filterpaper.

(b) Results and discussion.

The results are shown in Table II - 2.

From this table it can be seen that at 450°C, greater than 90% recovery was obtained for all three metals. At 500°C however, losses were evident. Since the recovery of zinc (Bpt. 907°C) was higher at 500°C than the recoveries of either copper (Bpt. 2595°C) or nickel (Bpt. 2732°C) it seemed unlikely that the losses were due to volatilisation of the metals. Alternatively the chlorides of these metals which are more volatile than the metals themselves may have been lost, but by inspection of the boiling points of $ZnCl_2$ (732°C), $CuCl_2$ (decomposes to CuCl which boils at 1490°C), $NiCl_2$ (sublimes; 973°C), it was apparent that loss by volatilisation was not the reason for the low recoveries.

Incomplete dissolution on addition of the acid to the ash was unlikely since the oxides of all these metals are soluble in acid, especially when present in a finely divided state.

TABLE II - 2

Percentage recoveries of 10 μg each of nickel, copper, and zinc after ashing at a) 450°C and b) 500°C for 12 hours in the presence of filter paper.

	Nickel		Copper		Zinc	
	450°C	500°C	450°C	500°C	450°C	500°C
Mean [+]	91.5	75.8	94.5	86.6	97.5	88.3
Std.Dev. [++]	2.0	5.0	1.0	1.5	3.0	9.0

[+] Mean of 12 samples

[++] 8 of the 12 samples lay within this deviation from the mean.

The most probable reason was that some nickel and copper was bonded strongly to the glass surface and could not be redissolved in acid. This problem had been encountered in preliminary studies where it was found that ashing in the absence of filter paper resulted in less than 50% recovery for each of the three metals. The presence of filter paper in the tubes, however, resulted in recoveries greater than 90% when ashing at 450°C. Further comments on the function of thefilter paper will be made in SECTION V. With plant ash, however, no loss by this mechanism was expected to occur and in view of the negligible losses due to volatilisation of the metals, the dry ashing method at 450°C to 500°C was adopted in preference to the wet ashing procedure. Dry ashing also had the advantage that it required no supervision during the period of ashing.

4. Dissolution and analytical techniques adopted for plant samples.

A flow sheet for the plant analysis procedure is shown in Figure II - 1. This method resulted in approximately 98% dissolution (assuming all carbonaceous material had been removed by ashing) and the remaining approximately 2% was shown to be almost entirely silica (emission spectrographic analysis) with traces of calcium, magnesium, manganese, sodium and aluminium also present.

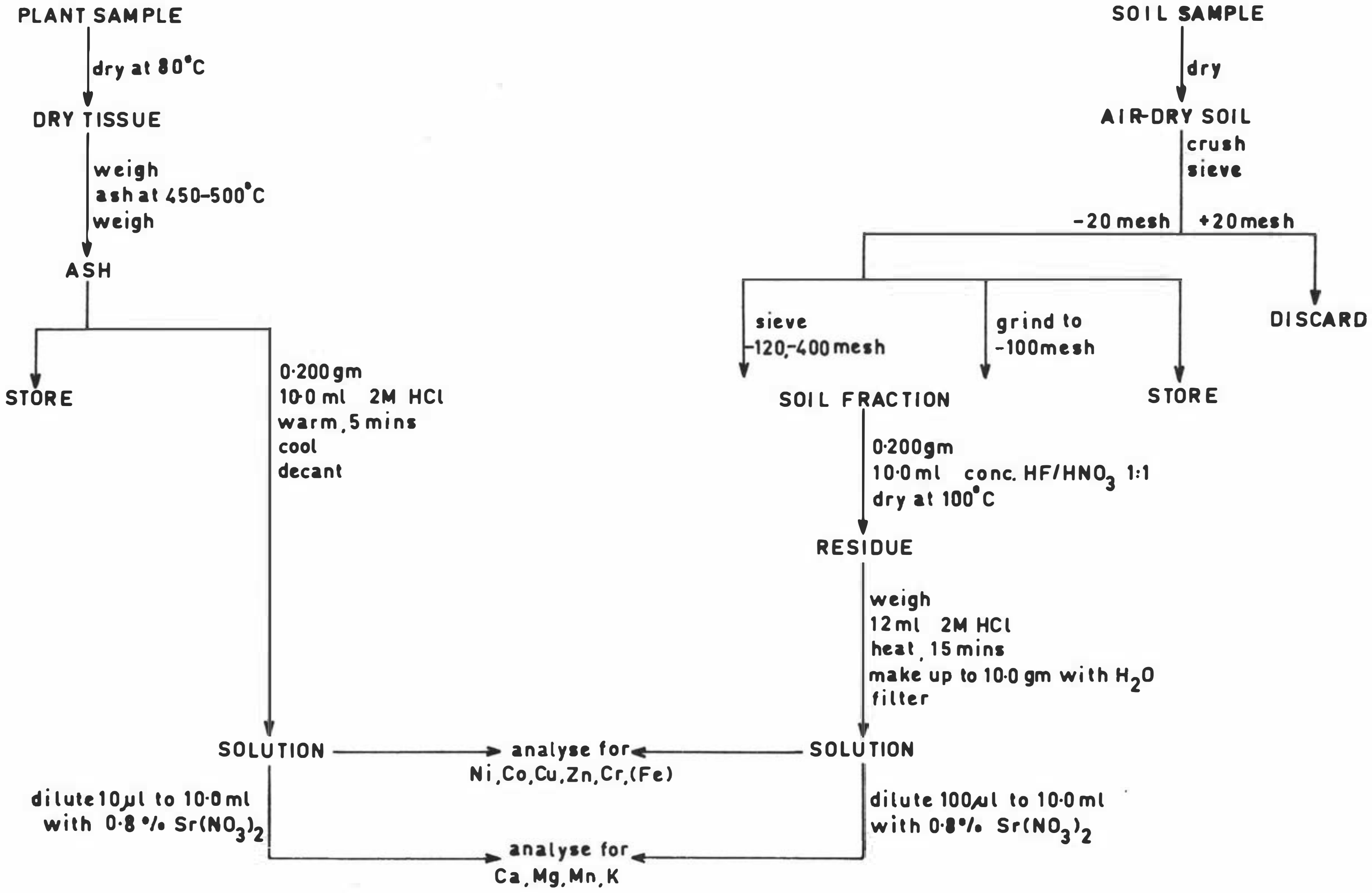


Figure II-1 Flowchart for plant and soil analysis.

D. TREATMENT OF SOIL SAMPLES.

1. Preliminary treatment.

Samples collected in the field were stored in plastic bag containers for transportation. The soils were air-dried in the laboratory, lightly crushed and sieved through a 20 mesh sieve. For the preliminary survey (SECTION III) total soil analyses were performed on the -20 mesh fraction after complete grinding by hand to -100 mesh.

2. Dissolution procedure.

There has been much discussion over the past years on the methods for dissolving silicate minerals. Ahrens et al.,(1963) described a detailed procedure based on ion-exchange and emission spectrographic techniques for the analysis of silicate rocks. This scheme used a hydrofluoric/aqua regia/sulphuric acid attack and gave complete dissolution although successive treatment was necessary for some samples.

Suhr and Ingamells (1966) described a solid fusion technique for the dissolution of silicate rocks using lithium tetraborate followed by dilute nitric acid. Solid fusion methods, however, are not readily applicable to the preparation of samples to be analysed for trace metals by atomic absorption spectrophotometry because of the high salt content arising from the fusion mixture.

Probably the most efficient method is a high temperature attack using concentrated hydrofluoric acid in a Teflon lined bomb (Langmyhr and Paus, 1968). While this method gives complete dissolution it is impractical if large numbers of samples are to be dissolved in a reasonable period of time with a minimum of man-hours. On the other hand, one of the most common acid mixtures used by mining companies for geological samples is that of concentrated perchloric/nitric acids. This does not, in general, give complete dissolution, but if the extent of the attack is the same for all samples, the results will be reasonably reliable.

In our laboratory, a modification of the procedure described by Brooks (1960) is used. Soil samples are attacked with concentrated hydrofluoric/nitric acids (1:1) in polypropylene beakers suspended in boiling water. The flow chart for the complete analysis by this technique is shown in Figure II - 1.

This procedure results in almost complete (≈99%) dissolution with the remaining metals in the residue being titanium and iron (presumably from magnetite and/or titanomagnetite) with lesser amounts of aluminium, silicon, calcium, magnesium, sodium and manganese. A very small amount of chromium was also detected (probably from chromite which is virtually insoluble under these acid conditions).

E. CHEMICAL AND SPECTRAL INTERFERENCES.

The high resolution of modern atomic absorption spectrophotometers makes interferences from spectral overlap virtually a problem of the past. Serious chemical interferences are also relatively few (Slavin and Slavin, 1969) with the chief problems arising in the measurement of the alkaline earths, calcium and magnesium (Govindaraju, 1970; Ramakrishna *et al.*,(1966)In plant samples most of the interference with the determination of these metals is caused by phosphate, while in soil solutions the interference arises mainly from aluminium and phosphate. Organic complexing reagents have been used to counteract this problem (Govindaraju, 1970; Wallace, 1963) but most of the interferences can be reduced to an acceptable level by adding excess strontium nitrate to the solution (Elwell and Gidley, 1967). In the present work, a solution of 0.8% $Sr(NO_3)_2$ (0.33%Sr) was used. Alkali metals can also cause interferences in the determination of calcium and magnesium due to ionisation effects (David, 1959) but these were largely overcome by using mixed standards of the metals.

F. THE ACCURACY AND PRECISION OF THE DISSOLUTION AND ANALYTICAL METHODS FOR PLANTS AND SOILS AND THE EFFECTS OF THESE PARAMETERS ON STATISTICAL TECHNIQUES.

1. Introduction.

The validity of any theory can only be as good as the validity of the evidence upon which it is based and in this thesis the validity of the conclusions and postulates are directly related to the error associated with the analytical results.

The question of error in geochemical sampling has been discussed by Miesch (1964). He separated the error into various components and commented on the effects these components had on different statistical procedures. His separation was as follows:

1) Bias - is the mean deviation of analytical results from their hypothetical "true" values. This error can be considered as the "accuracy" of the results.

2) Variable bias - is present if the bias varies with sampling position.

3) Variable precision - arises if the precision of the results is dependent on sampling position.

4) Precision - includes all variance due to sampling and analytical error.

In the classification of errors associated with data sets, No. 4 may be omitted because it is never zero, whereas the first three may or may not tend to zero for different sets.

With regard to statistical techniques such as trend analysis (see SECTION III E) and analysis of variance, both bias and variable precision can be tolerated in a data set without serious consequences. Variable bias, (variable accuracy) on the other hand, cannot be present to any great extent under any circumstances unless a quantative correction can be made for the variation. According to Miesch: "If type 3 error (variable bias) is very large and cannot be corrected, the data might as well be discarded."

It was concluded from these findings that for the purposes of the present thesis a reasonably precise knowledge was necessary of the following two points:

1) The precision of the analytical results since this can affect the stability of trend residuals. (Miesch, 1964) (see SECTION IV E) and

2) more important than precision, was the accuracy and the way in which this accuracy varied with the different samples.

2. Precision.

This was considered as the overall reproducibility of the dissolution and analysis scheme. To evaluate this parameter, five samples of the standard diabase W-1 were interplaced at random in a batch of approximately 100 soil samples. This procedure led to a true estimate of the precision actually obtained during a normal dissolution/analytical sequence. These results are shown in Table II - 3.

For all metals, the precision was good and adequate for this project since the variation between analyses of the same sample was far smaller than the variation observed between different samples.

Similar precision was obtained for plant samples.

3. Accuracy.

(a) Introduction.

Accuracy is the degree to which the true amount of a substance present in a sample can be determined.

Where a set of data is being evaluated on the basis of the relative magnitudes of each value within the set, as in this thesis, precision rather than accuracy is important, only if the relative accuracy for each measurement is constant (Miesch, 1964). That this is the case, is often assumed in routine atomic absorption analysis, for example, in the analysis of geochemical

TABLE II - 3

Analysis of the standard diabase W - 1.

	Metal	Sample					Ave	Accepted Value[x]	Deviation from Accepted[xx]	% Deviation
		1	2	3	4	5				
	Ca	7.25	7.25	8.00	7.75	7.25	7.50	7.80	-0.30	-3.84
	Mg	3.65	3.90	4.15	4.00	3.70	3.83	3.93	-0.05	-1.27
%	Mn	0.160	0.145	0.160	0.155	0.145	0.153	0.132	+0.021	+15.9
	K	0.525	0.510	0.525	0.500	0.500	0.512	0.523	-0.011	-2.10
	Fe	8.75	10.00	9.75	12.50	9.50	10.10	7.91	+2.19	+27.7
	Ni	108	101	103	105	101	103.6 (99.1)[‡]	78	+25.6	+32.8
	Co	78	75	80	78	78	77.8 (73.7)	50	+27.8	+55.6
p.p.m.	Cu	130	130	128	130	125	128.6 (117.9)	110	+18.6	+16.9
	Zn	110	101	108	135	105	111.8 (97.6)	82	+29.8	+36.3
	Cr	138	138	135	135	133	135.8	120	+15.8	+13.2

x Values from Fleischer (1965)

xx Values from concentration units

‡ From Fletcher (1970) on solutions diluted 0.100gm to 10.0ml.

prospecting samples. However, if interpretations (statistical or otherwise) are to be made of subtle variations in the data, then the relative accuracies must be considered.

From Table II - 3 it can be seen that the metals calcium, magnesium and potassium were all determined with very good accuracy (>96.0%). This was interesting because calcium fluoride is only slightly soluble and it has been suggested that boric acid be added to the solution to retain the calcium in soluble form. (Langmyhr and Paus, 1968). Manganese was found to be slightly higher than the established value but the error was acceptable.

When, however, the other transition metals were considered, serious inaccuracies were apparent.

In the case of iron, the error was caused mainly by acid contamination as mentioned previously but this could be corrected for by subtracting an appropriate acid blank. (This was not done in this case to illustrate the possible magnitude of the effect. Assuming the error was entirely due to contamination, the concentration of iron in the original concentrated acids was up to 4.48p.p.m.)

For zinc, some of the error was also due to this cause. The remaining error in zinc, and the error in the other metal results was almost certainly due in large part to the phenomenum known as "scattering".

When solutions of high salt concentrations (in the present case soil and plant solutions contained approximately 1.5% to 2.0%

solute) are aspirated into the flame of the atomic absorption spectrophotometer, solid particles form in the light path. These particles reflect the incident radiation from the lamp away from the slit causing an apparent absorption. Other workers have studied this effect in some detail (Billings, 1965; Koirtyohann and Pickett, 1966). Gidley (1964) studied the degree of scattering as a function of the wavelength, λ, and showed that it followed an approximately λ^{-4} dependence i.e. at short wavelengths the problem is greatest. That the particles are related to the major components in solution seems to be certain and Fletcher (1970) in his analyses of a number of geological standard rocks found similar errors to those observed in the present work (see Table II - 3). He made allowance for the scattering by aspirating artificial solutions containing the same concentrations of major cations as were in the standard rock solutions. The observed absorption was due to scattering.

For double-beam instruments Kahn (1968) described a system involving a deuterium lamp continuum which automatically compensated for scattering.

Since both instruments used in the present work were single beam, it was felt that it was necessary to know how the analytical accuracy was affected by scattering and how this accuracy varied with the concentrations of the major components in the plant ash and soil solutions.

(b) Methods.

For each of the cations present in high concentrations in normal plant ash and soil solutions, a series of pure solutions in 2 M hydrochloric acid were made to cover the range 0.02% to 2.0% cation concentration. "Specpure" salts were used for iron (Fe_2O_3), calcium ($CaCO_3$), magnesium (MgO), potassium (K_2SO_4) and sodium ($NaCl$), and B.D.H. analytical grade $Al(NO_3)_3.9H_2O$ was used for aluminium.

Each solution was aspirated at the analytical absorption line for the analytes lead, zinc, cadmium, cobalt, nickel and gold and the resulting absorption was compared with a standard of the analyte metal. For each of the cations tested for scattering, curves of cation concentration versus p.p.m. analyte due to scattering were constructed. These graphs are shown in Figure II - 2.

(c) Results and discussion.

It was apparent from the results obtained in this study and from the results obtained by Fletcher (1970) that in the analysis of trace metals in rock samples by atomic absorption spectrophotometry, errors of considerable magnitude could result. For nickel, cobalt and zinc, concentrations approximately 20p.p.m. too high were obtained while for chromium and copper the concentrations were too great by approximately 10p.p.m.

From the results presented in Figure II - 2 it can be seen that calcium and iron caused the most scattering for all the analyte metals investigated, although in some cases the other four cations made significant contributions also.

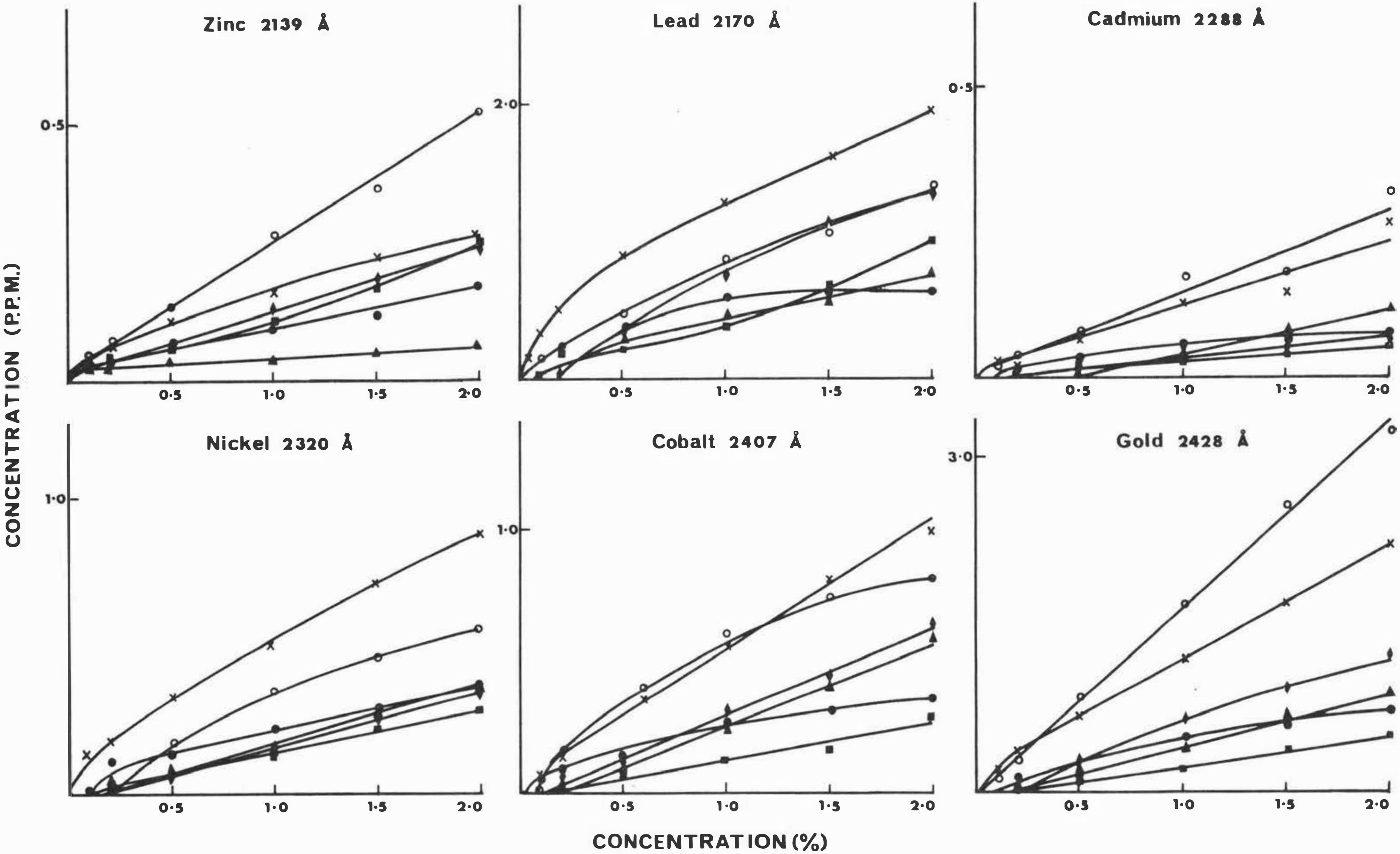


Figure II-2. Apparent concentrations due to light scatter by the following metals; calcium × iron ○ aluminium ● magnesium ■ potassium ♦ sodium ▲

These curves indicate the approximate extent of scattering. If for example, a plant ash containing 20% calcium is dissolved by adding 10.0ml of acid to 0.200gm of ash and this solution is analysed for cobalt, a signal corresponding to at least 0.2p.p.m. would be obtained, corresponging to 10p.p.m. cobalt in the original plant ash. This 10p.p.m. would be due entirely to scattering.

In the case of soils or rocks, the error in the cobalt determination was shown to be approximately 20p.p.m. If a soil apparently contained 50 p.p.m. cobalt (a normal value in the present study), 20p.p.m. of this would probably be due to scattering thus representing an error of +66% in the true concentration. Since the scattering was apparently due mainly to iron and calcium, and since the concentrations of these metals could vary by up to 50% between soil samples, then it is possible to have an error of up to ± 10p.p.m. in the 20p.p.m. due to scattering. This means a further error of ± 33% in the true concentration. The final result would contain an error from +33% up to +100%

(d) Conclusions.

From these results a number of conclusions were drawn:

1) Interference from scattering is most important for analyte metals with low sensitivity, whose absorption lines lie in the wavelength region less than 3000Å.

2) Out of the six metals tested, calcium and iron showed

the most interference but the other metals also contributed to varying degrees. In a normal plant ash or soil solution, however, it was not known whether the scattering was caused by each metal independently or whether binary or higher mixed salt particles were involved.

3) Since the major causes of scattering were found to be calcium and iron, the relative accuracies for different samples were dependent to a large extent on the way in which their calcium and iron contents varied.

For plant ash solutions, only calcium was expected to contribute significantly to scattering and since the concentrations of calcium in samples of the same species were very constant, then the relative accuracies were expected to be reasonably constant also.

Soil solutions, on the other hand, contained differing amounts of iron and calcium and the relative accuracies were expected to vary considerably.

4) In terms of the accuracy of the analytical data presented in this thesis, the following comments can be made:

(a) Copper and chromium - negligible interference was observed. The chromium concentrations in plant ash were very low but the scattering observed for plant ash solutions caused a signal equivalent to less than 3p.p.m. chromium in the plant ash.

(b) Zinc - most of the error observed was apparently due to contamination. The error due to scattering was considered to be insignificant.

(c) Nickel - errors of up to 25p.p.m. were observed for rock samples but since both plant ash and soil samples usually contained at least 100p.p.m. nickel, the error was able to be ignored.

(d) Cobalt - errors of approximately 30p.p.m. were observed for rock samples. Plant ash and soil samples contained apparent cobalt concentrations of approximately 20p.p.m. and 70p.p.m. respectively. Taking into account the low levels in the plants and the variation expected in the relative accuracies between soil samples, the measured cobalt concentrations in both plants and soils were not considered to be reliable, and except in one case no consideration is given to cobalt analyses in the following work.

5) Although most of the interference observed with concentrated solutions could undoubtedly be attributed to light scattering by particles, it seemed that other mechanisms, such as molecular absorption, might also be present.

G. MEASUREMENT OF SOIL pH.

There are a number of different ways of determining the pH of soils (Metson, 1956). Usually the air-dry soil is suspended either in a potassium chloride solution or in distilled water. In the present study, a mixture of 1:10 soil to water was used. Samples were shaken for six hours, allowed to settle overnight and then measured with a separate glass/calomel electrode system.

H. STATISTICAL PROCEDURES.

During the course of this project, large amounts of analytical data were obtained for up to ten metals in both plants and soils. A rapid method of detecting and evaluating relationships between data sets was necessary and resort was made to an IBM 1620II computer to calculate correlation coefficients and for multiple regression analysis.

Programmes in FORTRAN II-D were written by the author for this computer and a description of these programmes is included in the appendix.

Further discussion on the statistical techniques used will be presented prior to their use in later chapters.

SECTION III

PRELIMINARY BIOGEOCHEMICAL SURVEY

A. THE SAMPLING GRID.

Two grid areas of different sizes were used in this project and are illustrated in Figure III - 1. For the preliminary survey (SECTION III), 96 rectangularly distributed sites plus three additional sites downstream from the grid area were used, while for the major survey (SECTION IV) this grid was enlarged to contain 147 sites (see Figure III - 1). Grid coordinates given in this thesis contain the south coordinate followed by the east coordinate i.e. (south, east).

The altitude over the grid area ranged from 1500 feet to 2500 feet.

Indigenous forest covered the area (see Plate I) and further comment on the vegetation cover is made later in this section.

Rainfall was in the region of 80 to 100 inches per annum with the major part falling between March and September (de Lisle and Kerr, 1965).

The geological contact between the sedimentary rocks and the basic igneous rocks is shown in its approximate position in Figure III - 1. This was established on the basis of field observations and later soil analyses supported this position.

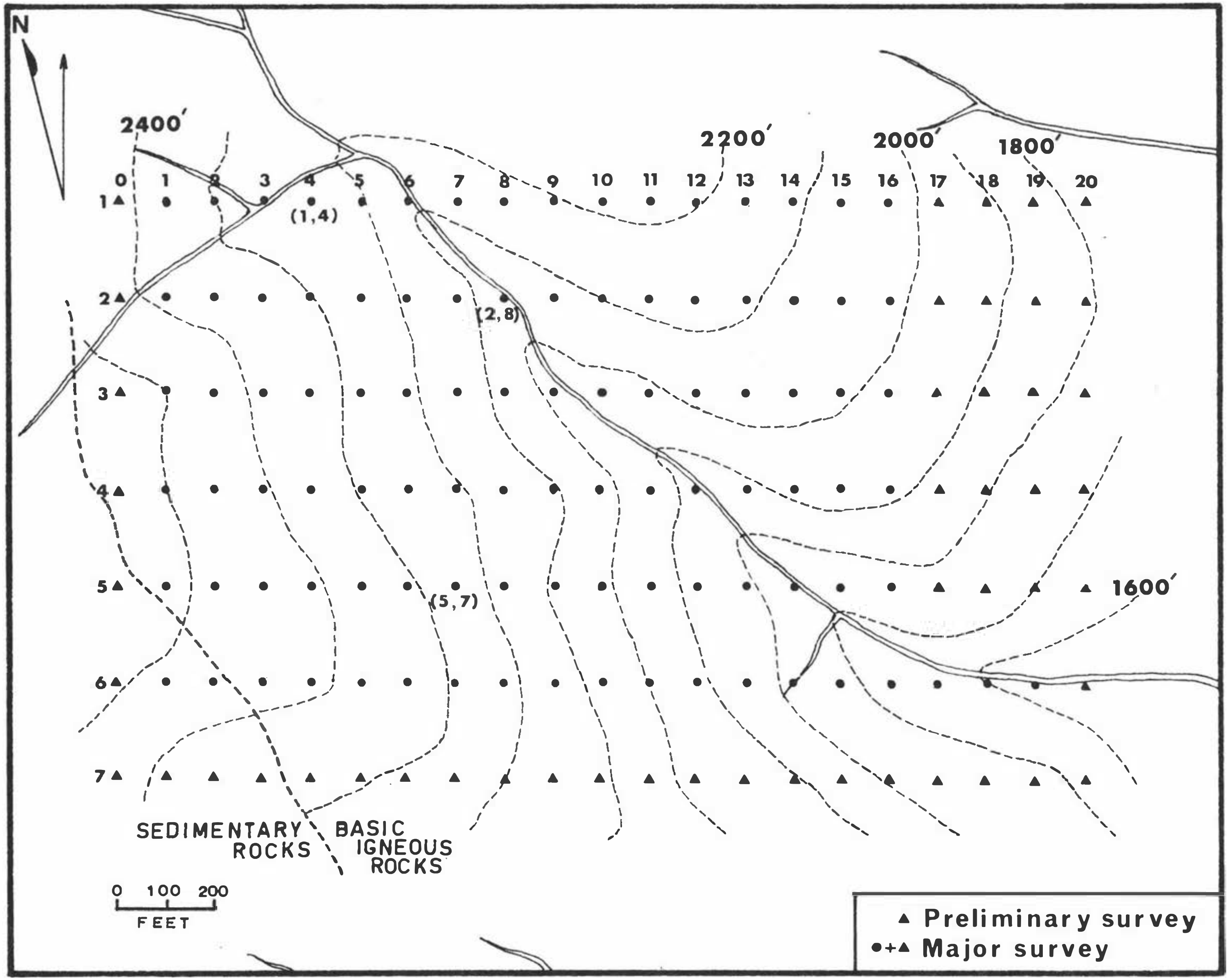


Figure III-1. The sampling grid.

PLATE I. Aerial photograph showing the topography and vegetation cover in the vicinity of the sampling grid. Upper-most clearing on the lower right is the camp site (3,1) and lower-most clearing is a drill pad at site (2,6).

B. MINERALISATION WITHIN THE SAMPLING GRID.

The only exposed mineralisation of note within the grid was found at site (2,8) (see Figure III - 1). This outcrop consisted of copper sulphides with minor nickel and contained approximately 1.3% copper and 0.2% nickel. Other similar but very minor areas of mineralisation were present near sites (1,4) and (5,7). These occurrences gave rise to anomalous soil concentrations of copper and nickel in their immediate vicinities and in their respective drainage basins. Thus, reasonably wide and well distributed ranges of nickel and copper concentrations were present in the soil.

C. VEGETATION COVER.

1. Introduction.

There were two main reasons for undertaking a comprehensive study of the vegetation cover in the area to be prospected.

Firstly, if samples were to be collected objectively then the particular species selected had to be widely distributed over the area to allow a regular sampling pattern. Problems of objective and subjective sampling and the merits of different sampling patterns have been discussed by Miesch *et al.*(1964).

Secondly, there was the possibility that geobotanical prospecting could be useful to indicate areas of copper or nickel mineralisation.

Two methods of studying plant distributions are in common use: aerial photography and ground plant mapping. Both were used in this thesis.

2. Aerial Photography.

(a) Introduction and methods.

The technique of measuring the vegetation balance from aerial colour and black and white photography has been used for many years, particularly as a means of evaluating forest reserves (Spurr, 1960).

In recent years a considerable amount of investigation into the use of aerial colour infrared photography has been carried out (Colwell, 1964, 1968; Fritz, 1967; Haack, 1962; Pease and Bowden, 1969). The film used for this type of photography has increased sensitivity in the range 7000 to 9000 Å when used in conjunction with a Wratten 12 or similar filter. In this wavelength range the colour differences between different species, and also between specimens of the same species which suffer from some type of stress, are enhanced relative to the differences in normal colour.

The varying infrared reflectances arising from specimens of plant species suffering from stress have been used for many years as a means of detecting various types of disease in crops or forests. Bawden (1933) found that potato streak virus damage appears darker in an infrared photograph than to the eye. Norman and Fritz (1965) observed that unhealthy citrus trees in Florida showed infrared reflectances ranging from magenta through purple to green depending on the particular disease infecting the trees. Healthy trees produced a reddish photographic rendition. A number of similar examples are given by Colwell (1956).

Ciesla *et al.*, (1967) and Stellingwerf, (1966) showed that yellow or orange spots in conifers, visible on colour infrared photographs, indicated attack on trees by the southern pine beetle or the Douglas fir beetle.

Evidence of stress in plants caused by excessive concentrations of divalent cations, particularly trace metals, in their substrates, has been available for some time. Forbes (1917) showed that excessive copper in the soil caused chlorosis in plants such as corn, beans and squash. DeKock (1956) showed that high concentrations of any of the cations - nickel, copper, cobalt, manganese, zinc and chromium - caused chlorosis in mustard plants. Hewitt (1948) also observed that excessive amounts of nickel, copper, cobalt and zinc caused chlorosis in sugar beet seedlings.

On the other hand, observations of extensive chlorosis caused by anomalous concentrations of copper and nickel in the soil are not generally observed under natural, undisturbed conditions. This is because such areas usually contain tolerant populations of plants. Although it is possible to observe chlorosis in the field where recent disturbances have occurred such as the redistribution of mineralised soil, no disturbance of any significance had occurred in the present area. It is not known, however, just how much disturbance in its foliar pigments a plant can tolerate over a long period of time. If different specimens of the same species can survive with slightly different ratios of their leaf pigments, chlorophyll, carotenoids and xanthophylls due to above-optimal concentrations of nickel or copper in the soil, then these differences may well be apparent from their infrared reflectances.

To look for species differences as well as evidence of stress due to mineralisation in the vegetation over the grid area, aerial photography was carried out using colour infrared photography (Kodak Ektachrome Infrared AERO Film, Type 8443) as well as normal colour photography (Kodachrome II).

(b) Results and discussion.

The results from both types of film showed a homogeneous forest cover over the sampling grid with an approximately constant ratio of species of the Nothofagus genus to Weinmannia racemosa. The colour infrared and to a much lesser extent the normal colour photographs showed an apparent change in the vegetation balance approximately 800 feet uphill (i.e. west) from the sampling grid. Ground observations showed this change to be caused by a sharp discontinuation in the presence of W.racemosa and an increase in the presence of Nothofagus menziesii (silver beech) relative to the other beech species N.fusca (red beech) and N.truncata (hard beech). This was probably due to the well known effect of altitude on this genus in New Zealand (Cockayne, 1926).

There was no apparent influence on the vegetation by the areas of anomalous concentrations of copper and nickel in the soil. Any minor effects, however, would have been virtually impossible to distinguish from the influences of topographical features, such as streams, aspect etc.

3. Ground Survey.

(a) Methods.

Plant mapping on the ground was undertaken to confirm the aerial photographic observations and also to check on the possibilty of understorey vegetation distributions being disturbed by anomalous metal concentrations in the soil.

There are a large number of different methods for measuring plant distributions, each suited to a different situation and to a particular purpose. (Goodall, 1952; Greig-Smith, 1964). The procedure used in the present survey was as follows: At intervals of 200 feet along a grid line running east-west, quadrats of 20 feet square were marked out and all the vegetation species, their trunk diameters and their frequencies of occurrence were noted for each quadrat. Two line transects were used, one from site (2,0) to site (2,20) and the other from site (7,0) to site (7,20).

(b) Results and discussion.

Apart from the observation that N.menziesii tended to predominate at higher altitudes and N.truncata at lower altitudes, there were no significant variations in the vegetation balance over the grid area. Any possible influences from geochemical factors were masked by the variation in topography, soil structure, drainage etc. between quadrats.

The species recorded in this mapping are listed in Appendix I.

D. EVALUATION OF PLANT AND SOIL SAMPLING PROCEDURES.

1. Introduction.

From the results of the aerial photographic and the plant mapping studies it was decided to sample the three species of the *Nothofagus* genus, *N.fusca* (Hook,f.) Oerst., *N.menziesii* (Hook.f.) Oerst. and *N.truncata* (Col.) Ckn., in addition to *Weinmannia racemosa* Linn.f. and *Quintinia acutifolia* Kirk.

The *Nothofagus* genus belongs to the Beech family *Fagaceae* and is the representative of this family in the Southern Hemisphere. *W.racemosa* and *Q.acutifolia* belong to the families of *Cunoniaceae* and *Escalloniaceae* respectively and both belong to the order *Cunoniales*.

Previous workers (Carlisle and Cleveland, 1958; Warren *et al.*, 1955) have shown that the concentrations of some metals in the leaves and twigs at different heights on a tree can vary markedly and Barakso (1969) has noted that metal concentrations can increase considerably with depth in the soil. It was therefore of importance that prior to the biogeochemical survey, some knowledge of the behaviour of different metals in the system was obtained.

There were three sources from which large errors could arise; the height on the tree and the depth in the soil from which samples were taken and the varying distances from the plants sampled at which the corresponding soil was sampled. Each of these three sources of error was investigated briefly.

2. Leaves, twigs and trunk wood sampled from different heights.

(a) Results.

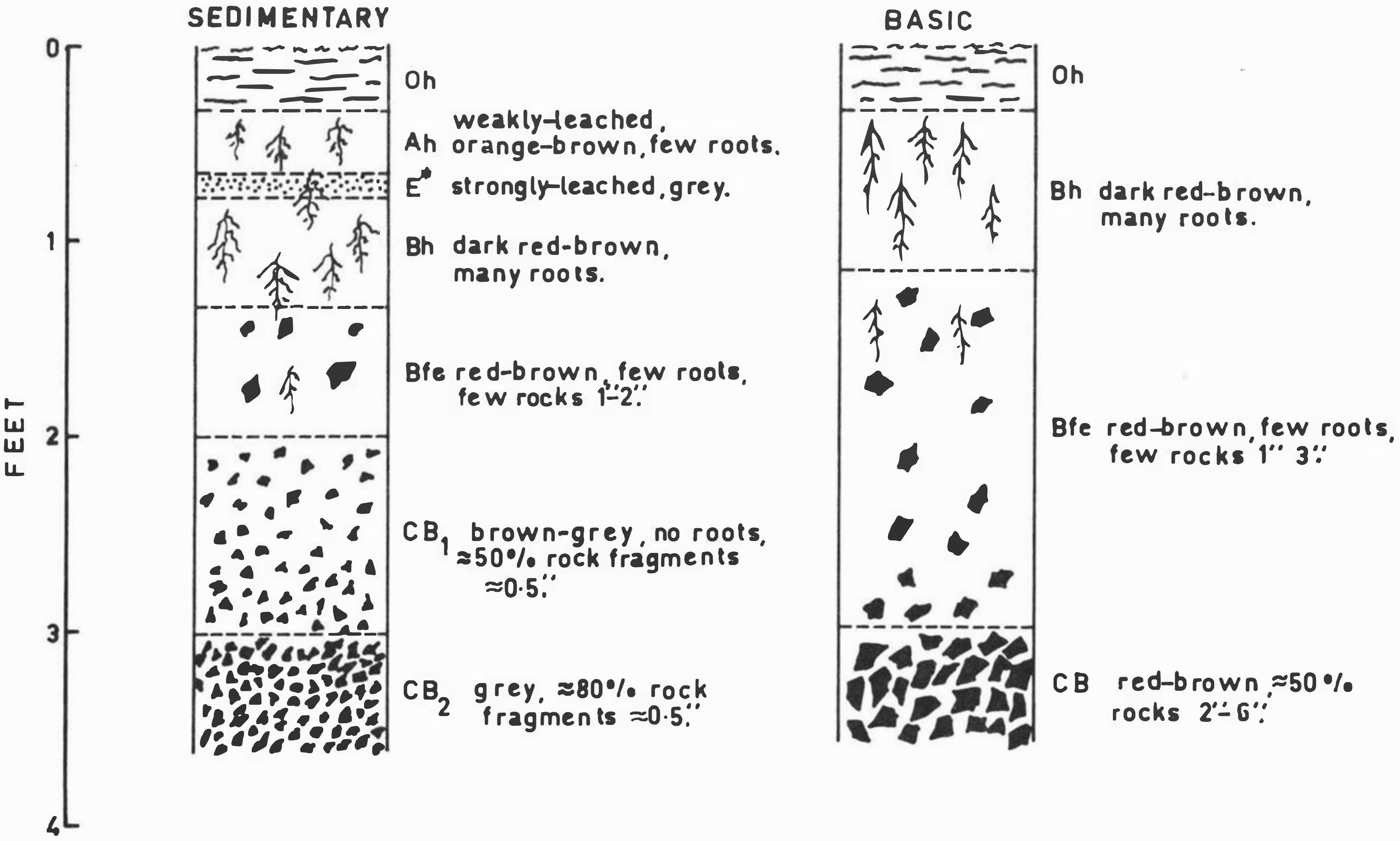
The analyses of these parts are presented in Table III - 1.

For most metals, the concentrations were not found to vary significantly between samples collected from different heights on the trees. There were, however, some apparent trends. The iron content in the leaf and twig ash showed a marked decrease with height for the taller of the two specimens but this behaviour was not evident for the other specimen. The concentration of zinc also showed a slight decrease with height in the leaves of the taller specimen. The manganese concentration showed a decrease with height which was apparent in both specimens while, on the other hand, the potassium concentration increased with height in both specimens. These effects were most pronounced in the taller of the two specimens.

3. Samples from different depths in the soil.

(a) Results.

Two types of soil were present in the area, one derived from sedimentary rock at the western end of the sampling grid, and the other derived from the basic rocks of the complex. Figure III - 2 shows typical profiles from each rock type and Table III - 2 gives the metal concentrations in the various horizons from two profiles on each parent rock.



*E horizon present in one profile only.

Figure III-2. Soil profiles. (horizon classification system from Corbett, 1969)

TABLE III - 1

Metal concentrations in ash of leaves, twigs and trunkwood taken from different heights up to two specimens of <u>Nothofagus fusca</u>.

+ A = Sampling height expressed as a fraction of the total height.

Total Height	Plant Part	A+	% Ash	p.p.m.					%			
				Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
80 feet	leaves	0.25	4.93	295	143	250	15	1050	19.0	4.10	1.30	8.10
		0.38	4.54	185	130	235	9	1050	18.5	6.00	1.03	8.90
		0.50	4.19	198	143	210	11	890	17.5	5.75	1.03	10.00
		1.00	4.69	175	115	190	7	730	18.0	5.25	0.68	10.00
	twigs	0.25	3.42	106	80	110	-	875	-	-	-	-
		0.38	3.24	154	51	120	-	625	-	-	-	-
		0.51	2.85	106	63	105	-	640	-	-	-	-
		1.00	4.69	125	46	100	-	490	-	-	-	-
38 feet	leaves	0.26	7.29	75	75	210	13	850	20.5	3.00	1.90	7.45
		0.53	6.45	75	65	290	13	950	20.0	3.25	1.75	7.70
		0.79	6.46	85	70	340	15	950	20.0	3.25	1.80	8.60
		1.00	6.45	65	65	310	13	700	19.5	4.00	1.35	8.30
	trunk wood	0.26	0.83	133	59	130	-	480	22.0	3.00	0.86	9.20
		0.53	1.24	105	60	300	-	390	24.0	2.90	0.87	6.60
		0.79	2.06	132	61	175	-	530	21.0	2.40	0.96	6.00

TABLE III - 2

Total metal concentrations at different depths in the soil.

Rock type	Site	Horizon	pH	p.p.m.				%				
				Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
Sedimentary	(6,0)	Oh	-	40	50	85	80	-	2.40	0.65	0.055	1.10
		Ah	4.90	50	30	70	140	4.10	0.30	0.90	0.030	3.30
		E	4.40	20	25	35	75	1.40	0.20	0.80	0.040	3.40
		Bfe	4.60	100	60	140	160	4.50	0.40	0.40	0.040	3.30
		CB	4.60	125	100	175	160	4.30	0.70	1.20	0.050	3.50
	(6,1)	Oh	-	15	30	40	45	-	0.55	0.50	0.040	0.60
		Ah	3.70	30	30	40	110	3.10	0.60	0.80	0.030	3.12
		Bh	4.15	40	40	60	125	3.40	0.55	0.80	0.025	2.95
		Bfe	4.30	95	55	130	135	4.30	0.50	0.70	0.035	3.12
		CB1	4.10	135	125	160	180	4.40	0.80	2.15	0.025	3.90
		CB2	3.70	150	115	175	205	4.40	1.15	2.40	0.060	3.56
Basic	(2,1)	Oh	-	55	35	40	110	-	1.35	1.25	0.045	0.20
		Bh	4.40	145	100	75	360	5.75	3.20	3.50	0.080	1.22
		Bfe	5.30	190	230	90	400	6.10	3.05	3.40	0.075	1.22
		CB	5.35	235	410	100	420	6.25	2.65	3.20	0.070	1.32
	(5,4)	Bh	5.40	180	105	100	400	7.90	6.90	5.25	0.015	1.62
		Bfe	5.00	185	150	110	400	8.30	6.20	4.80	0.105	1.62
		CB	4.90	210	200	120	420	8.50	6.00	5.40	1.105	1.72

The profiles derived from the sedimentary parent rock showed distinct A and B horizons and a transitional zone below the B horizons which was subdivided into two, mainly on the basis of the rock content and partly on the colour. In one profile an extensively leached horizon was recorded.

The two basic profiles studied showed less development than those derived from the sedimentary rock, with only three main horizons apparent. No extensively eluviated horizons were observed as was expected with a basic parent rock. Both humus and iron accumulation were evident above a horizon of closely packed rocks which was classified as a transition horizon between the illuviated horizons and the parent rock.

The most important observation from these studies was that most of the plant roots were confined to the upper two feet of the soil profiles.

For both types of soil, most of the metals analysed showed a general increase in concentration with depth, by up to a factor of five in some cases.

4. Variations of the metal concentrations in plants and soils over small distances.

(a) Methods.

A small area with an approximate diameter of 20 feet was studied, in which samples were collected of four soils and five saplings of N.fusca all with trunk diameters 0.5 inch to 1 inch (measured 1 foot above ground level) and heights between 6 feet and 8 feet.

(b) Results.

These samples were analysed for various metals and the results presented by taking an arbitrary origin and plotting the concentrations of these metals in the plants and soils at different distances from this origin (Figure III - 3). (Note: Although there are plant and soil samples at similar distances from the origin, they were collected at different points of the compass from the origin.)

These results showed that the nickel concentrations in the plants varied from 50p.p.m. to 150p.p.m. while in the soil the concentrations ranged from 100p.p.m. to 170p.p.m. The copper concentrations in the plants were remarkably constant while the copper concentrations in the soil ranged from 55p.p.m. to 325p.p.m. over the area.

The variations observed for the other metals were also large. When the range of variation for a particular metal was expressed as a percentage of the lowest concentration observed for the metal, the following results were obtained for the leaf ash (first value) and soils (second value); zinc, 133%, 33%; chromium, 50%, 7%; iron, 200%, -; calcium, 44%, 41%; magnesium, 108%, 25%; manganese, 110%, 13%; potassium, 14%, 12%. The pH of the soil

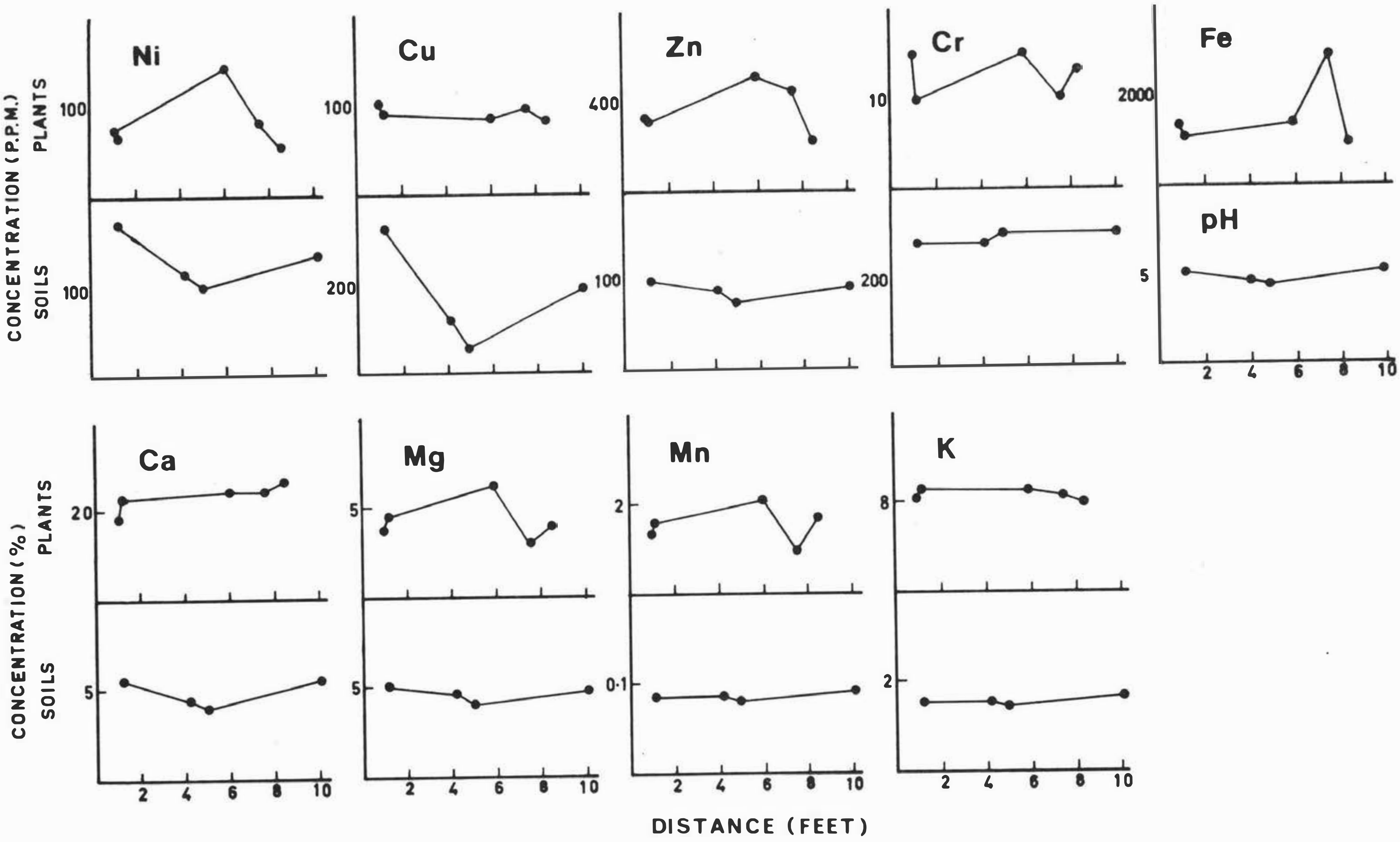


Figure III-3. Variations of the metal concentrations in plants and soils over small distances.

showed a variation of 17%. For all the metals except copper, the variation observed was greater in the leaf ash than in the soil.

5. Discussion and conclusions.

It was apparent from the results obtained that the errors caused by sampling leaves and twigs from different heights on the trees were relatively small when compared with the errors which resulted from sampling soil at different depths and different distances from the trees.

Of possible physiological interest was the inverse manganese to potassium ratio which was observed with increasing height. Even though these results are based on values from only two trees, the question raised is worthy of further attention.

From these studies it was apparent that over both the sediments and the basic rocks, the nickel and copper concentrations could increase by up to four or five times with depth in the soil profile. As well as this increase, there did not appear to be any fixed relationship between the nickel and copper concentrations in the upper horizons, and the concentrations of these metals in the lower horizons. Different results for these metals as well as for the other metals studied would therefore be obtained for soil samples collected at different depths.

It was noticed, however, that most of the plant roots were confined to the upper horizons of the soil profiles in both types of soil. This observation was in agreement to that made in Ghana by Greenland and Kowal (1960) who noted that in one acre of tropical forest, 85.5% of the roots supporting the vegetation were within one foot of the soil surface.

It was concluded that under the conditions which existed in the sampling area, soils taken from between 6 inches and 12 inches below the humus were reasonable representations of the plant substrates.

The variations which were apparent among the samples taken small distances apart were quite large and although the results were from only a small area, they were sufficient to indicate two points:

(1) That soils had to be sampled as close as possible to the plant roots if any meaningful relationship was to be obtained and

(2) that the data obtained would contain a large random error caused by the types of close-range variations observed above.

The following sampling procedure was adopted: Sufficient leaves, usually ¼lb to ½lb were collected to give a composite sample of old and new leaves. Twigs approximately 0.25inch to 0.5inch in diameter were sampled. Approximately 0.5lb of soil from 6 inches to 12 inches below the humus was collected from as close to the tree as possible.

E. STATISTICAL TREATMENT OF THE DATA.

1. Correlation Coefficients.

As a preliminary device for scanning large quantities of data, Pearson Product Moment correlation coefficients were calculated by computer. This particular coefficient is quite sensitive to the distributions of the data sets, requiring normally distributed data for complete mathematical validity. Norris and Hjelm (1961) have tested the "robustness" of the coefficient when applied to non-normal data. They found that, in general, if the data distributions did not depart too far from normality, then the resulting coefficient was quite valid. With badly skewed data, however, erroneous results were obtained.

With most geochemical data, badly skewed or even multi-modal distributions are the rule, rather than the exception. This arises because the analytical results which indicate mineralisation are usually an order of magnitude larger than the bulk of the data from non-mineralised samples. Thus two populations are present and this largely invalidates the Pearson Product Moment correlation.

However, in the present work this coefficient was calculated and the reasons were as follows:

(1) Over the particular area under study there were, for most metals, no regions of very high concentrations in either the soil or the plants relative to the rest of the sampling grid. This caused the data distributions to be skewed rather than bimodal.

(2) The skewness was readily reduced by transformation of the data to base ten logarithms.

(3) The correlation coefficient was used primarily as a

scanning device. Data sets showing good correlation coefficients were checked either graphically or as contour maps (although only in specific cases are these shown in this thesis) if further use of them was to be made. In general, all good relationships gave rise to good correlation coefficients, but some bad relationships also gave good coefficients due to the data being skewed or bimodal. The graphing and contouring procedures detected these latter cases.

The programme used was written by the author and is discussed in Appendix III. All data sets were transformed to base ten logarithms prior to computation of the correlation coefficients. Levels of significance used in this thesis were taken from Fisher and Yates (1957).

In addition to the correlation coefficients, the geometric means, standard deviations and reduced major axes were printed out.

2. The reduced major axis and graphing techniques.

The reduced major axis has been discussed by Imbrie (1956) with reference to its application in palaeontology for the study of relative growth patterns in populations of fossils. Its advantages over the line-of-best-fit, and regression lines were summarised as follows:

(1) It makes no assumptions of independence;

(2) It is invariant under change of scale:

(3) It is simple to compute;

(4) Results obtained from its use are intuitively more reasonable than corresponding results obtained from regression analysis.

While this statistic undoubtedly has advantages for the applications discussed by Imbrie, its general application to biogeochemical data is limited for the following reason. The slope of this line is given by the ratio of the standard deviations of the two data sets. It follows from this, that a reduced major axis can never attain zero slope unless the standard deviation of one data set is zero, or infinity. Standard deviations such as these are rarely encountered in experimental data sets.

A simple test of the validity of the line, is the consideration of the correlation coefficient for the data sets. Where this is very significant (approaching + or - 1.0), the reduced major axis is a good representation of the relationship, but where the correlation coefficient is insignificant, the reduced major axis is misleading, since although the ratios of the standard deviations may be near unity there is, in fact, no relationship between the data sets.

In the cases where good correlations were evident, however, the line was useful to illustrate graphically the relationships and it was for this reason that its calculation was included in the programme although no applications of the line are given in this thesis.

Graphs with large numbers of scattered points are presented in this thesis without any lines drawn on them. The author considers that in the absence of a mathematical approach, such as a least-squares-fit, the human eye is, in general, a better judge of two dimensional trends than the ruler. Correlation coefficients are presented on these graphs to illustrate the extents of the overall linear relationships.

F. PRELIMINARY SAMPLING PROGRAMME.

1. Metal concentrations in the leaves and twigs of the species sampled.

(a) Results.

The results of the plant analyses are presented in Table III - 3 and Figure III - 4. At this stage, the three species of *Nothofagus* were considered together. The most outstanding feature of these results was the differences that existed between the concentrations in the different species, not only of the trace metals but also of the major nutrients.

The mean metal concentrations in the soils corresponding to the plants are also presented in Table III - 3. These results show that the observed differences between species were not due to varying soil concentrations. The mean percentage ash in the dry tissue of each species is shown in the same table. Although these differ, the amount is not great enough to account for the observed variations in the results. This is illustrated by the dry-weight concentrations also shown in Table III - 3.

(i) Leaves.

It can be seen from this table and from Figure III - 4, that for the metals nickel, copper, zinc and iron, the highest concentrations tended to be in the leaves of the *Nothofagus* species, while for chromium and magnesium, the lowest concentrations occurred in the leaves of this genus.

The two species *W.racemosa*, and *Q.acutifolia* contained similar amounts of nickel, copper, zinc and magnesium. On the

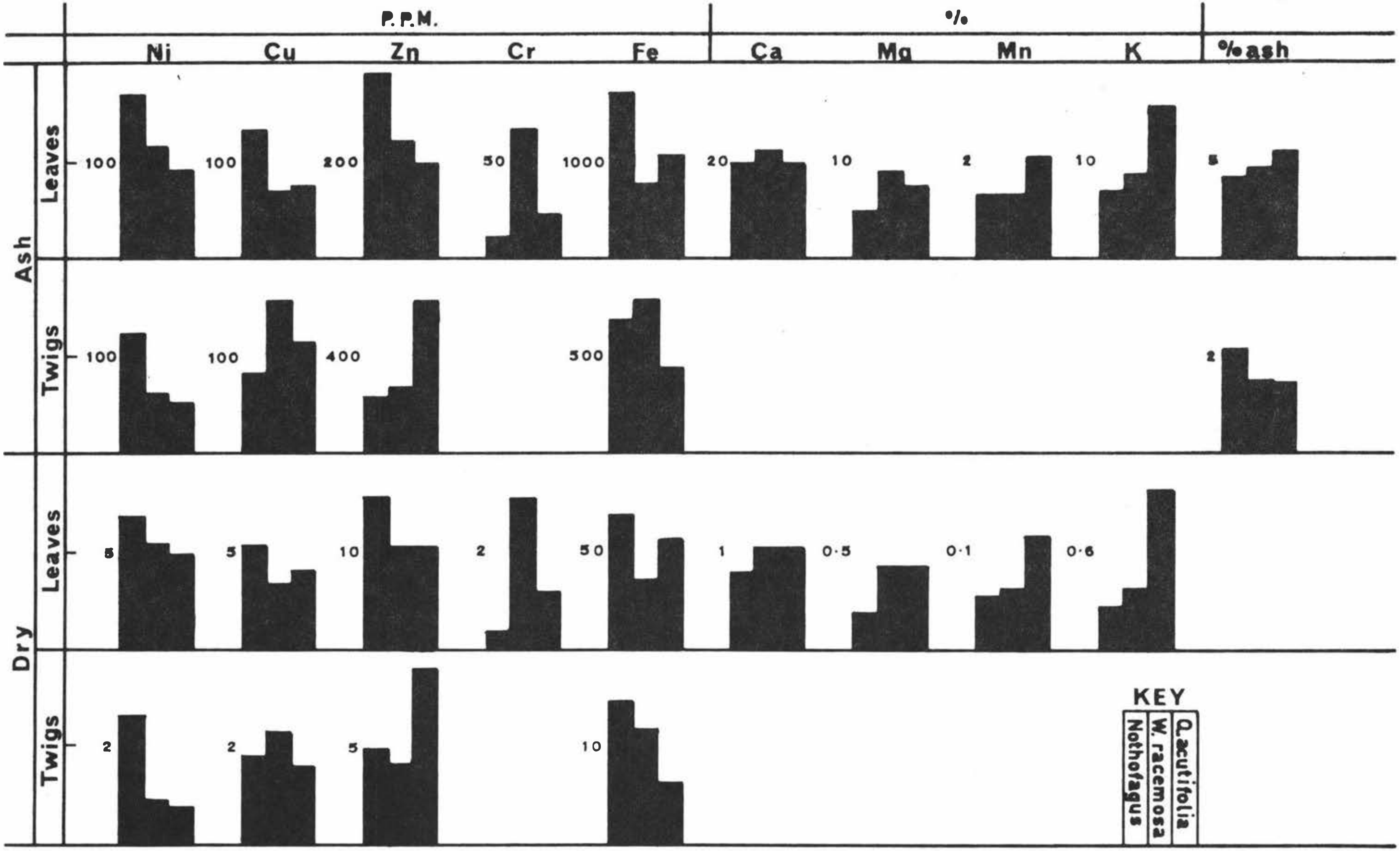


Figure III-4. Metal concentrations in the plants sampled.

TABLE III - 3

Mean metal concentrations in the leaves and twigs of the plants sampled.

Species	No. of Samples	% Ash	p.p.m.					%			
			Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
Leaves - Ash											
Nothofagus spp	86	4.05	166	130	377	9.90	1701	19.2	4.65	1.28	6.91
Soil			161	83.6	77.5	476	-	5.23	4.41	0.116	0.444
W. racemosa	94	4.65	113	69.5	218	66.4	754	22.0	8.84	1.29	8.04
Soil			151	76.6	77.1	462	-	5.14	4.29	0.114	0.446
Q. acutifolia	83	5.50	88.9	72.7	191	20.9	1035	19.1	7.37	2.07	17.7
Soil			149	74.5	77.0	464	-	5.22	4.34	0.148	0.435
Leaves - Dry Matter											
Nothofagus spp	86	4.05	6.72	5.28	15.3	0.402	69.1	0.780	0.189	0.053	0.281
W. racemosa	94	4.65	5.25	3.24	10.2	3.09	35.1	1.02	0.412	0.060	0.374
Q. acutifolia	88	5.50	4.89	4.00	10.5	1.14	56.9	1.05	0.411	0.116	0.973
Twigs - Ash											
Nothofagus spp	84	2.16	121	83.1	230	-	689				
Soil			180	92.8	87.6	-	-				
W. racemosa	95	1.46	61.7	156	275	-	787				
Soil			173	89.6	87.8	-	-				
Q. acutifolia	88	1.43	51.8	111	616	-	437				
Soil			169	85.1	87.8	-	-				
Twigs - Dry Matter											
Nothofagus spp	84	2.16	2.62	1.79	4.95	-	14.9				
W. racemosa	95	1.46	0.900	2.30	4.01	-	11.5				
Q. acutifolia	88	1.43	0.740	1.59	8.80	-	6.25				

N.B. Soil sample locations correspond to the plant sample locations.

other hand, the leaves of Q.acutifolia contained nearly twice as much manganese and three times as much potassium as the leaves of the other species.

Chromium, although present in only small amounts, showed striking differences between the species with concentrations ranging by a factor of six over the three plant types. The increase in the chromium content from the Nothofagus species through Q.acutifolia to W.racemosa was balanced by an inverse relationship for the iron concentration.

Calcium contents were similar for the leaf ash of all species, but on a dry weight basis the concentration was lower in the Nothofagus genus and in this respect calcium resembled magnesium for this genus.

(ii) Twigs.

Although the twigs were analysed for only nickel, copper, zinc and iron, some distinct trends were apparent.

For copper, the concentration was found to be highest in the twigs of W.racemosa in contrast to the concentration in the leaves where this species recorded the lowest value.

This behaviour was also evident for zinc, where Q.acutifolia contained the lowest concentration in its leaf ash but by far the highest concentration in its twig ash.

For iron, a similar trend was observed.

Nickel, on the other hand, showed the same inter-species relationship for the twigs as was observed for the leaves.

(iii) Relative accumulations.

The results are presented in a different form in Table III - 4 where the relative accumulation values (concentration in the plant tissue divided by the concentration in the soil) are given. From this table the difference between the species can be seen more clearly. The two most obvious features are the greater accumulation by *N.acutifolia* of potassium in its leaves and zinc in its twigs. Also the eightfold increase in the accumulation of chromium by *W.racemosa* relative to the *Nothofagus* genus is worthy of note.

The accumulations of copper and zinc in the leaves and twigs showed an inverse relationship among the three plant types as was observed for the absolute concentrations.

(b) Discussion.

The results presented above illustrate two features concerning the balance of metals in plants. Firstly, the different relative amounts of metals in the leaves of the various species and, secondly, the way in which trace metals are distributed between the leaves and the twigs of a particular species.

TABLE III - 4

Mean relative accumulations of the metals in the leaves and twigs of the plants sampled.

Species	No. of Samples	% Ash	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
Leaves - Ash										
Nothofagus spp.	86	4.05	1.03	1.56	4.86	0.0208	3.67	1.05	11.0	15.6
W.racemosa	94	4.65	0.745	0.906	2.83	0.144	4.28	2.06	11.3	18.0
Q.acutifolia	88	5.50	0.597	0.976	2.48	0.0450	3.66	1.70	14.0	40.6
Leaves - Dry Matter										
Nothofagus spp	86	4.05	0.0417	0.0631	0.197	0.00084	0.149	0.0425	0.445	0.631
W.racemosa	94	4.65	0.0346	0.0422	0.132	0.00670	0.199	0.0958	0.525	0.836
Q.acutifolia	83	5.50	0.0320	0.0524	0.136	0.00248	0.201	0.0935	0.770	2.23
Twigs - Ash										
Nothofagus spp	84	2.16	0.672	0.896	2.62					
W.racemosa	95	1.46	0.357	1.74	3.14					
Q.acutifolia	88	1.43	0.306	1.30	7.02					
Twigs - Dry Matter										
Nothofagus spp	84	2.16	0.0145	0.0194	0.0565					
W.racemosa	95	1.46	0.00521	0.0254	0.0458					
Q.acutifolia	83	1.43	0.00438	0.0186	0.100					

The relative amounts of different metals in the leaves of the different species were most markedly illustrated by the chromium and iron concentrations; where the iron content was high, the chromium content was low and vice versa. Iron is known to be essential to plant nutrition (Bowen, 1966) but although chromium is now known to perform a necessary function in the glucose tolerance of mammals (Mertz, 1970; Schroeder, 1968), its essentiality to plant growth has not been unequivocally demonstrated. The interrelationship between these two metals observed in the present study, although not indicating that chromium is related to the metabolic function of iron, does seem to point to its accumulation being, in some way, linked to the uptake of iron.

Apart from the generally higher concentrations of nickel, copper and zinc in the leaves of the Nothofagus species, the other outstanding finding was the relatively high level of manganese and potassium in the leaves of Q.acutifolia. This species had a much larger leaf area than the other species and it is possible that the contents of these metals are related in some way to this factor. Further comment on this observation is made in a later section.

The distributions of copper and zinc between the leaves and twigs of the different plants were of considerable interest. Where the accumulation of either of these metals tended to be low in the leaves it tended to be high in the twigs and vice versa. This finding could indicate that although approximately constant contents of copper and zinc were maintained in the plant tissue, the actual distributions of these "pools" of metals varied between the leaves and the twigs of the different species. Q.acutifolia may have less storage capacity in its leaves for copper and zinc and more in its young twigs relative to the other species. This suggestion, however, is quite speculative and it would require a study of the metal distributions at a sub-cellular level to confirm or disprove it.

The concentrations of the major nutrient metals calcium, magnesium and potassium in the species studied in this thesis are compared in Table III - 5 to the concentrations found in some other species. It must be pointed out that comparisons with the metal contents of trees growing in different areas can only be very approximate because of the widely different soil types usually involved. Some of the results quoted in Table III - 5 from other workers include values for plants growing on soils deficient in essential metals whereas, to the author's knowledge, none of the values presented by other workers were obtained in basic soils as was the case in the present thesis.

In general, the values obtained in this thesis are in good agreement with the values reported by Miller (1963) for *N.truncata* and by Peterson (1962) for *Agathis australis.* Comparisons with the other trees in Table III - 5 indicate that the *Nothofagus* genus was more similar to the conifers, (*Pinus* species) than to the other deciduous trees *Q.acutifolia*, *W.racemosa* and *B.verrucosa*. Miller (1963) made a similar conclusion with respect to *N.truncata*.

The levels of trace metals in the trees of New Zealand are not extensively documented. Comparisons can, however, be made with the trace metal contents of trees in other parts of the world if it is borne in mind that different soils are involved in most cases.

TABLE III - 5

Mean concentrations and concentration ranges of calcium, magnesium and potassium in the dry leaves of different trees.

Species	No. of Samples	Data+ Source	Ca	Mg	K
Nothofagus spp.	86	A	0.78	0.19	0.28
Nothofagus truncata	1	B	0.4-0.8	0.12-0.18	0.3-0.6
Agathis australis	35	C	0.43-1.75	-	0.14-0.90
W. racemosa	94	A	1.02	0.41	0.37
Q. acutifolia	88	A	1.05	0.41	0.97
Betula verrucosa	8	D	1.57	0.27	1.56
Pinus sylvestris L.	-	E	0.4-0.6	0.07-0.16	0.5-1.1
P. radiata	3	F	0.12	-	0.90
P. resinosa	3	G	0.3-0.6	0.12-0.16	0.4-0.9

+

A This thesis

B Miller (1963)

C Peterson (1962)

D Ovington and Madgwick (1959)

E Ovington (1959)

F Orman and Will (1960)

G Madgwick (1964)

Lounamaa (1956) quotes typical figures for deciduous trees and shrubs growing over soils derived from ultrabasic rocks. In the leaf ash, nickel ranged up to 3000p.p.m. while the typical value for copper was 94p.p.m.,for zinc 1200p.p.m., for chromium 26p.p.m. and for manganese 1.5%. Twig ash contained similar concentrations of nickel,130p.p.m, copper,2700 p.p.m. and 1.1% manganese in the ash.

The values obtained in this thesis are in good agreement with the values presented by Lounamaa for all metals except zinc. In the present study zinc concentrations ranged from 200p.p.m. to 400p.p.m. in the leaf ash and 200p.p.m. to 600p.p.m. in the twig ash in contrast to the much higher values given by Lounamaa. Warren (1962) presented zinc concentrations ranging from 200p.p.m. to over 4000p.p.m. in the ash of the twigs from a large number of plant species growing in British Columbia. On the other hand, copper and zinc concentrations reported by Peterson (1962) for the New Zealand indigenous species *Agathis australis* were within the same concentration ranges observed for the species studied in this thesis.

The above observations suggest that even in view of the fact that different soils were involved in the comparisons, the New Zealand indigenous trees studied in this thesis contained lower concentrations of zinc than trees in other parts of the world.

2. The relationships between the metal concentrations in the plants and in the soil.

(a) Results.

The evaluation of the plant~~x~~soil

relationships for all the metal/plant species/plant part systems was achieved by computing correlation coefficients on both an ash and a dry weight basis. These results are given in Table III - 6.

The Nothofagus species, W.racemosa and Q.acutifolia all showed highly significant (<0.001) correlations between the concentration of nickel in the leaves and the concentration of this metal in the soil. In the case of copper, however, only the Nothofagus species and Q.acutifolia showed a highly significant relationship between the concentration in the leaves and the concentration in the soil. For both these metals similar although less significant results were obtained for the twigs.

In the case of chromium in the leaves, highly significant correlations were observed for W.racemosa and Q.acutifolia in both the ash and the dry leaves and significant (<0.01) correlations were apparent for the dry leaves of the Nothofagus species.

Magnesium in Q.acutifolia leaves was the only other metal to show a highly significant plantxsoil relationship.

(b) Discussion.

If one considers both nickel and chromium at the concentrations in these plants to be non-essential to plant nutrition, then it is unlikely that a mechanism will be present to regulate the accumulation of these metals unless potentially toxic levels are present. Therefore, the amounts of

TABLE III - 6

Correlation coefficients between the metal concentrations in the plants and in the soils.

		Leaves									Twigs		
	Species	No. of Samples	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K	No. of Samples	Ni	Cu	Zn
Ash	*Nothofagus* spp.	85	0.562^{++}	0.375^{++}	0.138	0.203	-0.199	0.161	-0.104	0.034	84	0.412^{++}	0.291^{+}	-0.051
	W. racemosa	94	0.427^{++}	0.054	0.016	0.650^{++}	0.094	0.253	-0.109	0.242	95	0.515^{++}	0.067	0.013
	Q. acutifolia	88	0.411^{++}	0.355^{++}	0.0007	0.562^{++}	0.225	0.481^{++}	-0.018	0.099	88	0.327^{+}	0.338^{++}	0.082
Dry	*Nothofagus* spp.	85	0.707^{++}	0.587^{++}	0.138	0.340^{+}	0.002	0.162	-0.109	-0.034	84	0.444^{++}	0.251	-0.024
	W. racemosa	94	0.432^{++}	0.034	0.016	0.635^{++}	0.032	0.253	-0.044	0.242	95	0.457^{++}	0.127	0.116
	Q. acutifolia	88	0.368^{++}	0.369^{++}	0.0007	0.539^{++}	0.076	0.482^{++}	-0.005	0.099	88	0.113	0.120	-0.019

Levels of significance ++ < 0.001

\+ < 0.01

these metals in the plant tissues would be expected to reflect the fluctuations in the available contents in the soil. On the other hand, it was apparent that for the remaining metals, except copper and magnesium, the concentrations in the plant tissue were regulated by the plants themselves and not by the soil.

In the case of copper and magnesium, although these metals are essential for plant nutrition, it appeared from the results obtained that the accumulation was, in fact, influenced to a small extent by the soil. This was most evident for copper in the *Nothofagus* genus and for both copper and magnesium in *Q.acutifolia*.

In the soils of the area studied, the copper concentrations varied considerably, due to the presence of copper mineralisation. Magnesium also varied considerably over the area due to the varying concentrations of this metal in the parent rocks. While it was expected that the plants were capable of accumulating essential metals to meet their requirements, it was apparent that where the available concentrations in the soil greatly exceeded the levels required, the plants could not restrict their accumulation of these metals. In the extreme case, although not observed in the present study, the concentrations become toxic or the plants evolve a tolerance mechanism (Peterson, 1971).

The reasons why *W.racemosa* showed a non-significant plant x soil relationship for copper in its leaves is not known, but because the mean soil concentration of this metal was similar for this species (77p.p.m.) as for the other species (74p.p.m and 83p.p.m) it must be concluded that W.racemosa has a more strict control of the levels of copper in its leaf tissue. The concentration of copper in the leaf tissue of W.racemosa was lower than for the other species. This difference between the species may seem surprising, but when it is viewed alongside the vast differences that existed between the metal accumulations and distributions in the leaves and twigs of the different species, it becomes rather more credible.

These findings have important implications for biogeochemical prospecting. If the concentrations of metals in the soils are to be predicted from the concentrations in plant tissue, then highly significant correlations between these two variables will indicate a promising plant/metal system for prospecting purposes.

As can be seen from Table III - 6, the Nothofagus genus showed the most potential for the prediction of copper and nickel concentrations in the soil. These statistical results were checked in the case of nickel by plotting the isoconcentration contour maps for the leaf ash shown in Figure III - 5, A, B, C. The merits of the Nothofagus species were clearly evident. However, all species gave good correlations for the case of nickel and when the areas of anomalous concentrations common to all species were considered, good agreement with the soil anomalies was found. (Figure III - 5D) The use of many plant species, to indicate nickel soil anomalies, was of considerable value to reinforce the findings, but the additional labour involved made this technique unattractive.

The isoconcentration contour map for the copper concentration in the leaf ash of the Nothofagus species is shown in Figure III -6.

Figure III - 5 Iso-concentration contours of nickel in both the plants and the soils.

A *Nothofagus* species

B *W.racemosa*

C *Q.acutifolia*

D Areas of anomalous nickel concentrations common to all species.

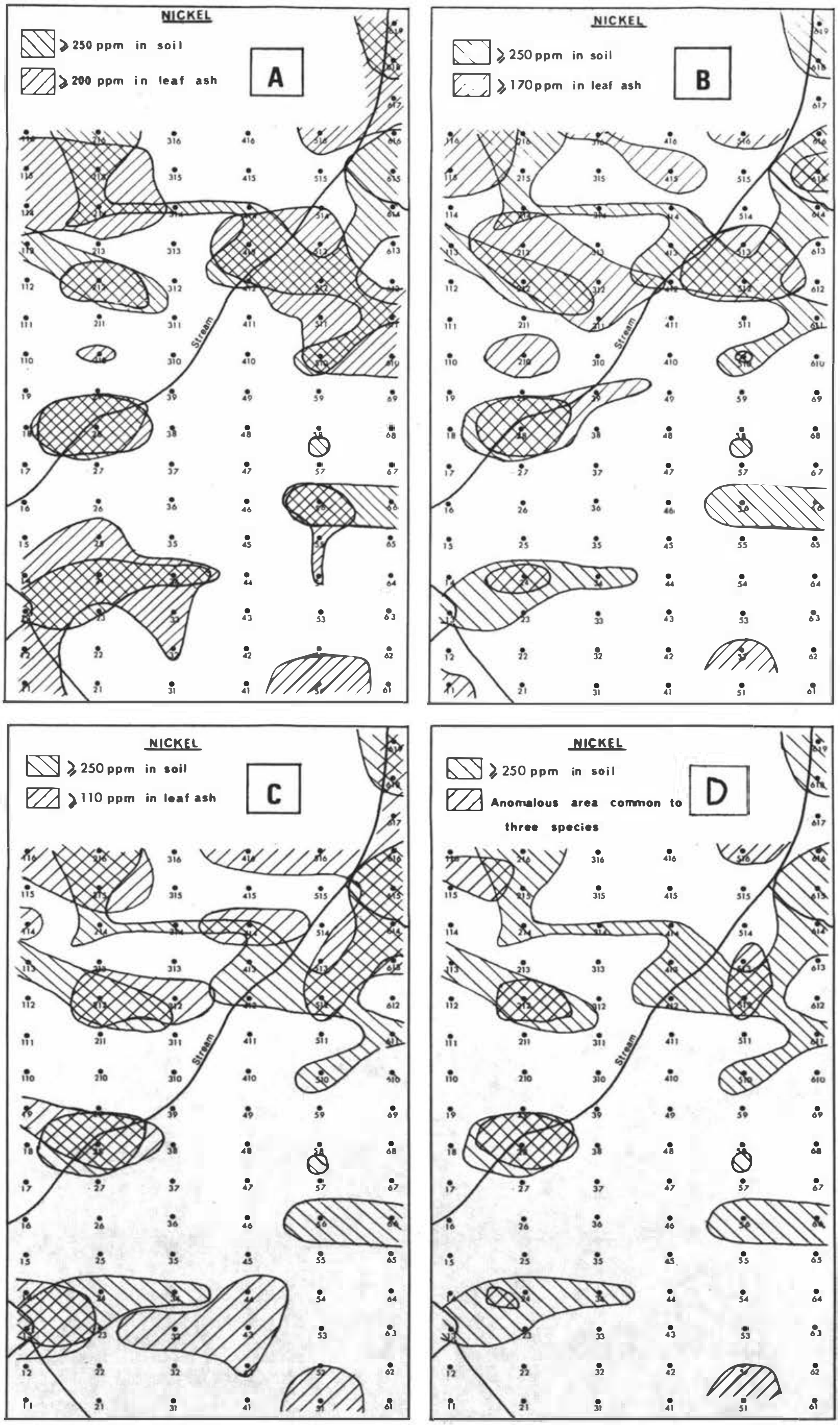

NICKEL
A
> 250 ppm in soil
> 200 ppm in leaf ash
B
> 250 ppm in soil
> 170 ppm in leaf ash
C
> 250 ppm in soil
> 110 ppm in leaf ash
D
> 250 ppm in soil
Anomalous area common to three species
Stream

Figure III-5.

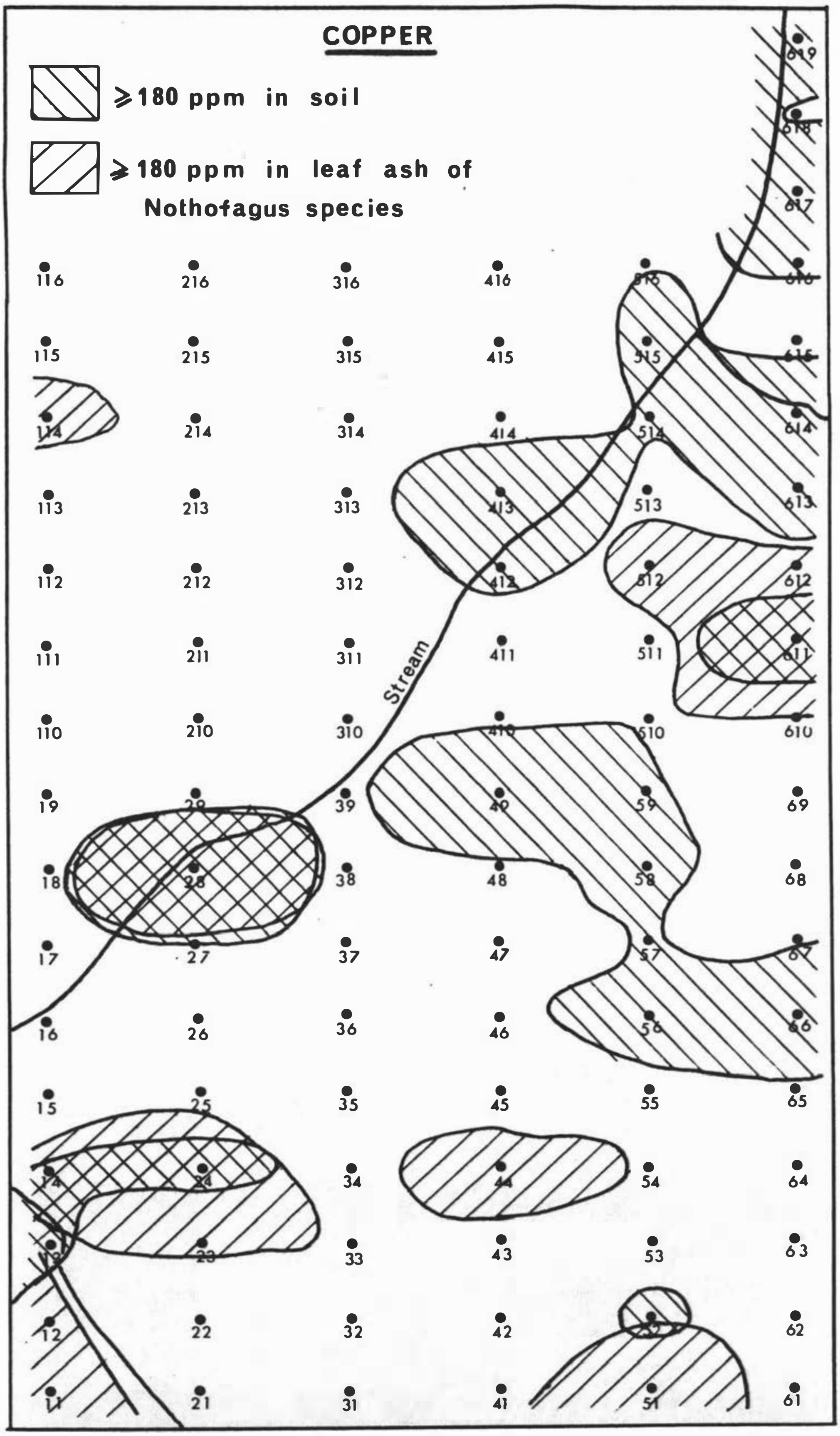


Figure III-6. Iso-concentration contours of copper in both the *Nothofagus* spp. and the soil.

This plant/metal system did not show such a good relationship as that observed for the case of nickel.

Although the correlation coefficients for the nickel and copper concentrations between the twig ash and the soils were similar to those obtained for the leaf ash, it was decided to sample leaves in preference to twigs because firstly, they were easier to collect and secondly, on ashing, the leaves produced a higher percentage ash than did the twigs.

The relative merits of data expressed on a dry weight or an ash weight basis were difficult to evaluate. The results presented above indicated that dry weight data was preferable, but since the differences between the results on an ash and a dry weight basis were not great, it was decided to use ash weight data preferentially.

More extensive work (SECTION IV-D) showed that the differences between the two types of data were not as great as indicated by the above results.

Although the findings of this study showed that the species of the Nothofagus genus were the most promising to study further, it was necessary to know what effect different proportions of the three species N.fusca, N.menziesii and N.truncata would have on the results of a biogeochemical prospecting survey.

3. The three Nothofagus species.

(a) Introduction.

The samples collected previously were grouped according to species and the mean metal concentrations for each group were determined. It was found that the percentage ash weights were different for each species, so for comparative purposes, concentrations on both a dry weight and an ash weight basis are presented.

(b) Results.

The mean concentrations for the metals analysed in the leaves of the Nothofagus species are presented in Table III - 7.

From these results it can be seen that the leaf ash of N.menziesii contained the highest concentrations of all metals except calcium. It was noticed, however, that the percentage ash in N.menziesii was considerably lower than that for the other two Nothofagus species and when the metal concentrations in the dry material were compared it was found that there were only small differences between the three species with the exception of the zinc, iron and calcium concentrations.

Since the mean metal concentrations in the soils corresponding to each species were different, further comparisons of the concentrations of the metals between species were difficult.

To partially overcome this difficulty, the relative accumulation

TABLE III - 7

Mean metal concentrations in the leaves of the three *Nothofagus* species.

Species	No. of Samples	% Ash	Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
			p.p.m.					%			
ASH											
N. fusca	35	4.99	152	113	256	8.14	1261	18.7	4.63	0.110	6.09
Soil			182	107	77.5	510	-	5.52	4.68	0.120	0.424
N. truncata	10	4.96	150	88.8	221	7.32	1016	20.7	4.10	0.976	5.47
Soil			204	62.5	73.7	847	-	6.40	5.41	0.107	0.392
N. menziesii	41	3.23	183	161	596	12.6	2492	19.3	4.81	1.55	8.15
Soil			137	73.2	78.4	390	-	4.75	3.99	0.114	0.477
DRY MATTER											
N. fusca	35	4.99	7.58	5.64	12.8	0.406	63.0	0.933	0.231	0.0549	0.304
N. truncata	10	4.96	7.45	4.40	11.0	0.363	50.4	1.03	0.203	0.0484	0.271
N. menziesii	41	3.23	5.91	5.20	19.3	0.407	80.5	0.623	0.155	0.0500	0.263

TABLE III - 8

Mean relative accumulations for the metals in the leaves of the three *Nothofagus* species.

Species	No. of Samples	% Ash	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
ASH										
N. fusca	35	4.99	0.835	1.06	3.28	0.0159	3.39	0.993	9.18	14.4
N. truncata	10	4.96	0.737	1.42	2.99	0.0086	3.23	0.758	9,11	14.0
N. menziesii	41	3.23	1.40	2.26	7.52	0.0316	4.04	1.23	13.5	17.2
DRY MATTER										
N. fusca	35	4.99	0.0416	0.0526	0.163	0.0008	0.169	0.0496	0.458	0.719
N. truncata	10	4.96	0.0365	0.0704	0.149	0.0004	0.160	0.0376	0.452	0.692
N. menziesii	41	3.23	0.0454	0.0733	0.244	0.0010	0.131	0.0397	0.437	0.558

values were calculated and are presented in Table III - 8. These values show that when ash weight data are considered, N.fusca and N.truncata are more similar to each other than to N.menziesii. On the other hand, the relative accumulations expressed on a dry weight basis, showed little variation between the species with exception of the values for chromium and zinc. N.menziesii accumulated approximately twice as much zinc on a dry weight and an ash weight basis than did either N.fusca or N.truncata. Chromium was accumulated to a greater extent in the leaves of N.menziesii than in the leaves of N.fusca or N.truncata. The only marked differences that were apparent between N.truncata and N.fusca were in their copper and chromium accumulations. N.truncata accumulated more copper and less chromium than N.fusca relative to the total soil concentrations.

In Table III - 9, the relative accumulations for the various metals in N.truncata and N.menziesii are expressed relative to those for N.fusca. It can be seen from these results that, taking all the relative accumulations for the various metals in N.fusca as 1.0, the relative accumulations for all the metals in N.truncata are very close to 1.0 on both an ash weight and a dry weight basis with the exception of copper which was higher and chromium and magnesium which were lower than 1.0. On the other hand, the values for N.menziesii were all higher than 1.0 on an ash weight basis, and on a dry weight basis the trace metals nickel, copper, zinc and chromium were also higher than 1.0 but the other metals were lower than 1.0.

TABLE III - 9

Mean relative accumulations for N.truncata and N.menziesii expressed relative to the values for N.fusca.

Metal	N.truncata		N.menziesii	
	ash	dry	ash	dry
nickel	0.881	0.878	1.68	1.08
copper	1.34	1.34	2.13	1.39
zinc	0.912	0.915	2.29	1.50
chromium	0.541	0.508	1.99	1.25
calcium	0.953	0.948	1.19	0.775
magnesium	0.764	0.758	1.24	0.800
manganese	0.992	0.988	1.47	0.955
potassium	0.972	0.964	1.19	0.755

(c) Discussion and conclusions.

The differences between the ash percentages for *N.truncata* and *N.fusca* on one hand and *N.menziesii* on the other could be related to the physical characteristics of the species. *N.fusca* and *N.truncata* are quite similar in appearance whereas *N.menziesii* is distinctly different. (See Plate II) It was also of interest to note that the physical similarities between *N.fusca* and *N.truncata* were paralleled by their accumulations of nickel, zinc, calcium, manganese and potassium.

Further discussion concerning the metal concentrations in these species and the occurrence of hybridisation will be presented in a later section of this thesis.

It was concluded from the above results that since the relative accumulations of nickel by *N.fusca* and *N.truncata* were approximately the same on an ash weight basis (0.835 and 0.737, respectively) whereas that for *N.menziesii* was higher (1.40), it was preferable to sample *N.fusca* and *N.truncata* rather than *N.menziesii*. In the case of copper, since the three species showed different relative accumulations, there was no particular advantage in sampling one species in preference to another.

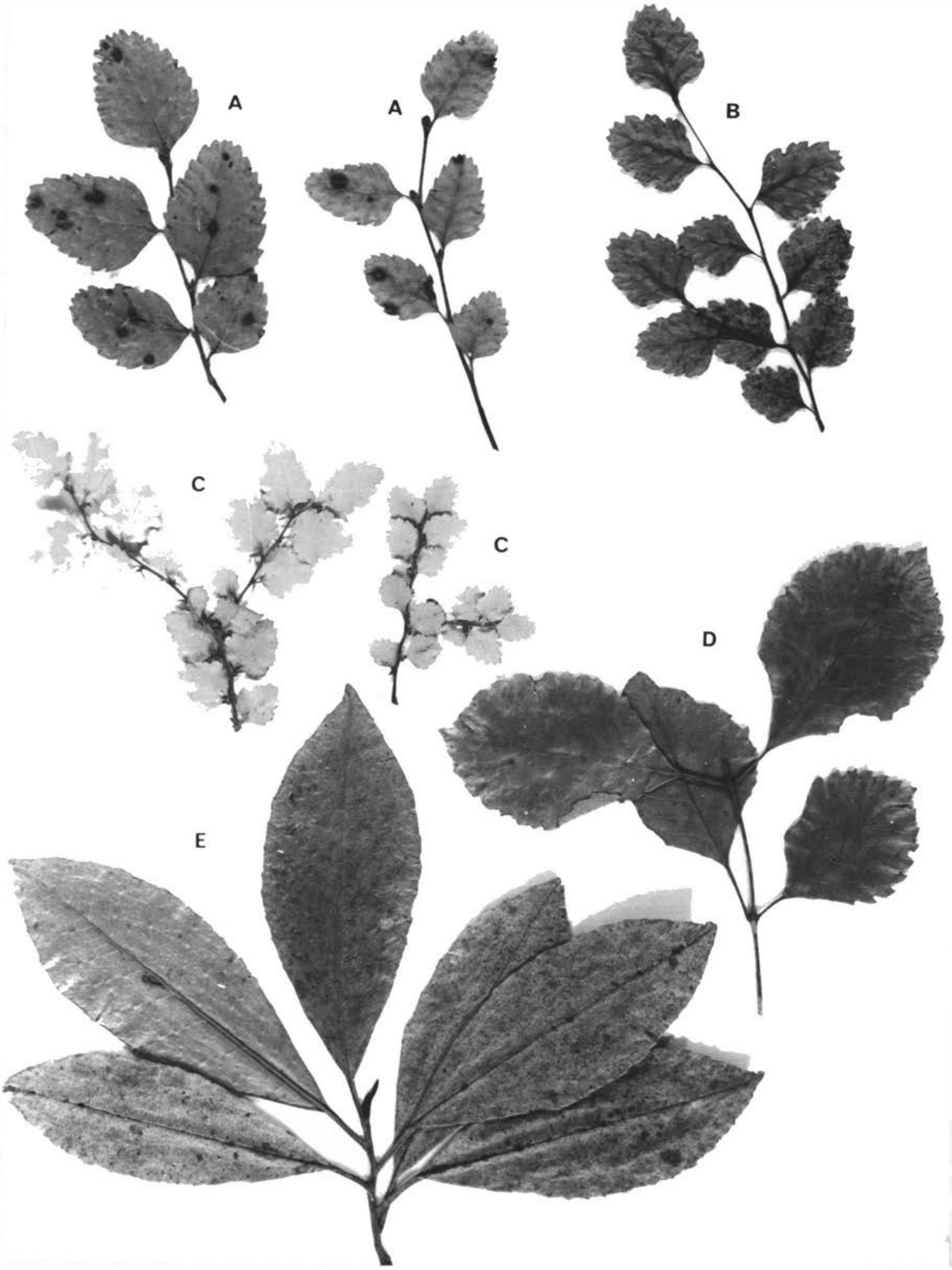


PLATE II. Leaves from the trees sampled.

A. Nothofagus truncata

B. N.fusca

C. N.menziesii

D. Weinmannia racemosa

E. Quintinia acutifolia

SECTION IV

MAJOR BIOGEOCHEMICAL SURVEY

A. INTRODUCTION.

The present section describes the results of a detailed investigation into the nature of the relationships between the metal concentrations in the leaves of the *Nothofagus* genus and the concentrations of these metals in the soils. Special consideration was given to copper and nickel to evaluate the useful of biogeochemical prospecting for these two metals. A method for the quantitative evaluation of factors affecting these relationships is also described.

The grid area used in the preliminary survey (SECTION III) was enlarged from 96 to 147 sites to encompass more of the anomalous areas and to provide a larger number of samples for statistical evaluation of the data. (see Figure III - 1)

The bulk of the sampling programme was carried out in November, 1969, and additions were made in November, 1970. It was considered that any seasonal differences would be small relative to the differences between different samples. Nine samples from the March, 1969 survey were added to complete the data set. The species balance in the final data set was *N.fusca*, 60 samples, *N.menziesii*, 33 samples, and *N.truncata*, 47 samples. Soils were taken at the same time as the corresponding plant samples.

B. THE PRECISION OF GEOCHEMICAL AND BIOGEOCHEMICAL SAMPLING.

1. Introduction.

In SECTION III, the magnitude of the sampling error associated with different aspects of the sampling procedure was investigated. The results obtained for a small number of samples showed that the errors inherent in both plant and soil sampling techniques were quite large. With a greater number of samples, however, the relative magnitudes of the error may be quite different between plant and soil sampling methods.

The resampling of both the Nothofagus genus and the corresponding soils from the grid sampled previously, allowed a comparison of the precisions of both plant and soil sampling procedures to be made. Seventyfour plant sites and 86 soil sites were common to both the November, 1969 and the March, 1969 sampling programmes and these were compared by correlation coefficients for all the metals as well as by graphs for copper and nickel.

2. Results.

The mean metal concentrations in the plants and soils are presented in Table IV - 1 and the correlation coefficients between the data decks of the two sampling surveys are shown in Table IV - 2.

Nickel, copper, zinc and iron all showed higher concentrations in the ash of the leaves collected in March whereas the other metals showed higher concentrations in the ash of the leaves

TABLE IV - 1

Mean metal concentrations in the plants and soils collected in March, 1969 and November, 1969.

Data Set		Survey	No. of Samples	% Ash	p.p.m.					%			
					Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
Nothofagus spp. leaves	ash	March	74	4.03	159	126	381	10.0	1695	19.3	4.59	1.35	6.87
		November	74	4.66	105	107	341	11.3	1297	21.4	5.00	1.45	10.9
	dry	March	74	4.03	6.41	5.08	15.4	0.403	68.4	0.778	0.185	0.055	0.277
		November	74	4.66	4.90	5.00	15.9	0.527	60.5	0.998	0.233	0.068	0.508
									pH				
Soils (-20 mesh)		March	86	-	179	88	88	550	5.39	5.34	4.47	0.115	0.438
		November	86	-	174	72	87	576	5.42	5.85	5.46	0.122	0.654

TABLE IV - 2

Correlation coefficients between samples collected in March, 1969 and November, 1969.

Data set	No. of Samples	Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
<u>Nothofagus</u> spp. leaf ash	74	0.380^{++}	0.144	0.320^{+}	0.038	0.466^{++}	0.072	0.329^{+}	0.419^{++}	0.324^{+}
						pH				
Soils (-20 mesh)	86	0.624^{++}	0.701^{++}	0.639^{++}	0.319^{++}	0.349^{++}	0.857^{++}	0.790^{++}	0.787^{++}	0.578^{++}

Levels of significance ++ < 0.001

\+ < 0.01

collected in the November survey. Similar results were observed for the dry leaves.

The concentrations of the metals in the soils followed the same trends as shown by the plants but in the soils the differences between the sampling surveys were not as great as the differences observed for the plants.

The correlation coefficients were, in general, less significant for the plant data than for the soil data. Only the nickel, manganese and iron concentrations in the leaf ash showed highly significant (<0.001) correlation coefficients between the two sampling surveys whereas all the metal concentrations in the soil, as well as the pH, were highly correlated between the surveys.

Because of the relevance of these results to prospecting for nickel and copper, graphs of the plant and soil data for the two surveys are shown in Figure IV - 1 for these two metals. It can be seen from these graphs that although the soil data show considerable scatter they were more closely related between the two surveys than were the plant data.

3. Discussion.

It was evident from the results presented above that the precision of the plant sampling procedure was very low in the particular area studied.

Figure IV - 1 Relationships between the data collected in the March, 1969 and November, 1969 sampling programmes.

A Nickel in soils (-20 mesh fraction)
B Copper in soils (-20 mesh fraction)
C Nickel in *Nothofagus* spp. leaf ash
D Copper in *Nothofagus* spp. leaf ash

N.B. Correlation coefficients (r) are shown on the graphs.

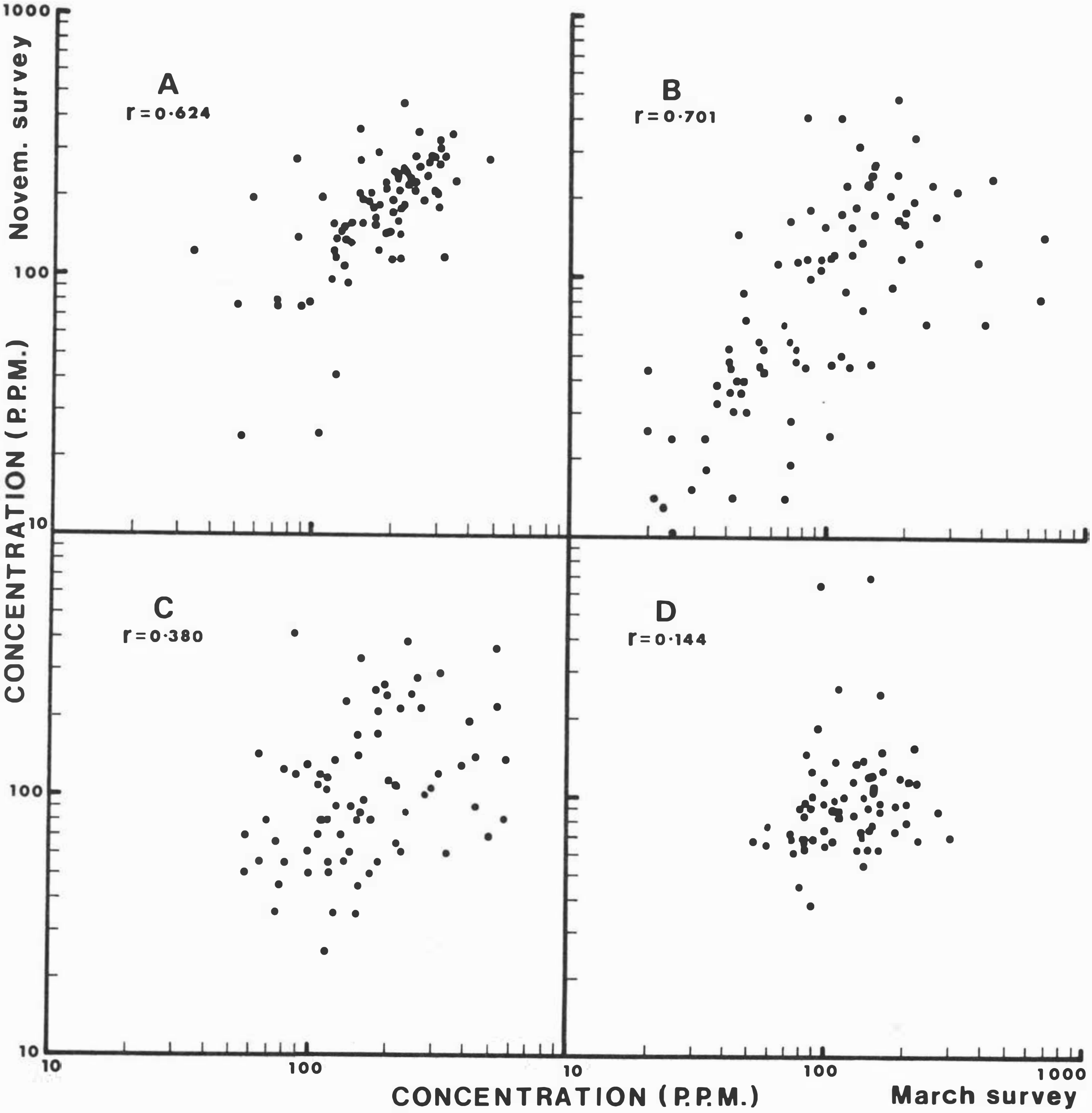

A
r = 0·624
B
r = 0·701
C
r = 0·380
D
r = 0·144
Novem. survey
CONCENTRATION (P.P.M.)
1000
100
10
10
100
10
10
100
10
100
1000
CONCENTRATION (P.P.M.)
March survey

Figure IV-1.

It should be pointed out that no attempt was made to resample the same individual trees nor was any attempt made to resample soil from precisely the same spot as that previously sampled. It is probable that the lack of agreement between plant samples was due to different trees being sampled in each survey, as well as to the natural variations in the metal contents of the leaves due to age, foliar leaching, changing light conditions beneath the forest canopy etc.

The soil samples showed close agreement between the surveys and this is a very definite point in favour of soil sampling relative to plant sampling.

One way of improving the precision of plant sampling would be to sample an area by means of "cells" rather than discrete sampling sites. To do this, the area is divided into small rectangles (cells) and a number of trees are sampled at random from each cell. The samples from each cell are analysed and the results averaged to give a value for the cell as a whole.

This procedure would overcome much of the variability among individual plant samples and should result in greater sampling precision. This precision could be increased further by taking a greater number of samples from each cell.

C. FURTHER COMMENTS ON THE THREE NOTHOFAGUS SPECIES.

1. Introduction.

In SECTION III it was shown that the percentage ash weights and the relative accumulations of most metals in *N.fusca* were more similar to the values for *N.truncata* than to the values for *N.menziesii*. In view of the possibility that these observations were related to physiological differences between the species, confirmation of the previous results (SECTION III-F) was sought from the present results.

Since the previous samples were collected in March, 1969 whereas the present samples were collected in November, 1969 seasonal differences were expected to be evident.

2. Results.

The mean percentage ash weights and the mean metal concentration on an ash and a dry weight basis are presented in Table IV - 3. As was observed previously (SECTION III-F) the mean percentage ash weight for *N.menziesii* was much lower than for either *N.fusca* or *N.truncata*. The percentage ash weights of the latter two species were quite similar, in agreement with the previous results.

The concentrations of all the metals were highest in the leaf ash of *N.menziesii*, but on a dry weight basis the concentrations of nickel, copper, zinc, chromium and iron were higher in this species.

TABLE IV - 3

Mean concentrations of some metals in the leaves of the three *Nothofagus* species collected in November, 1969.

			p.p.m.					%			
Species	No. of Samples	% Ash	Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
ASH											
N.fusca	35	4.90	85.6	99.7	309	10.3	1088	19.9	5.24	1.46	11.2
Soil			163	95.7	93.4	453	-	5.05	4.90	0.122	0.692
N.truncata	30	5.24	110	84.4	261	10.3	1101	22.0	4.27	1.30	9.18
Soil			185	39.7	77.7	909	-	6.53	5.90	0.113	0.615
N.menziesii	21	3.44	170	214	652	16.1	2273	22.7	5.90	1.71	13.4
Soil			178	93.3	90.5	475	-	6.37	5.85	0.136	0.667
DRY MATTER											
N.fusca	35	4.90	4.19	4.89	15.1	0.505	53.3	0.975	0.257	0.0715	0.549
N.truncata	30	5.24	5.76	4.42	13.7	0.540	57.6	1.15	0.224	0.0681	0.481
N.menziesii	21	3.44	5.84	7.36	22.4	0.554	73.0	0.780	0.203	0.0588	0.461

TABLE IV - 4

Mean relative accumulations of some metals in the leaves of the three *Nothofagus* species collected in March, 1969, and November, 1969.

Species	Survey	No. of Samples	% Ash	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
ASH											
N. fusca	March	35	4.99	0.835	1.06	3.23	0.0159	3.39	0.993	9.13	14.4
	Nov.	35	4.90	0.525	1.04	3.31	0.0227	3.94	1.07	11.9	16.3
N. truncata	March	10	4.96	0.737	1.42	2.99	0.0086	3.23	0.758	9.11	14.0
	Nov.	30	5.24	0.596	2.13	3.35	0.0112	3.36	0.724	11.5	14.9
N. menziesii	March	41	3.23	1.40	2.26	7.52	0.0316	4.04	1.23	13.5	17.2
	Nov.	21	3.44	0.959	2.29	7.20	0.0339	3.57	1.00	12.6	20.1
DRY MATTER											
N. fusca	March	35	4.99	0.0416	0.0526	0.163	0.0008	0.169	0.0496	0.453	0.719
	Nov.	35	4.90	0.0257	0.0511	0.163	0.0011	0.193	0.0524	0.586	0.797
N. truncata	March	10	4.96	0.0365	0.0704	0.149	0.0004	0.160	0.0376	0.452	0.692
	Nov.	30	5.24	0.0312	0.111	0.176	0.0005	0.176	0.0379	0.603	0.782
N. menziesii	March	41	3.23	0.0454	0.0733	0.244	0.0010	0.131	0.0317	0.437	0.558
	Nov.	21	3.44	0.0330	0.0789	0.243	0.0011	0.123	0.0345	0.433	0.691

Because of the differences that were evident between the mean metal concentrations in the soils corresponding to each species, the relative accumulation values for some metals gave a better basis for comparison between the species. These values are presented in Table IV - 4 as well as the values obtained in the previous survey.

On an ash weight basis, the relative accumulations obtained for both sampling surveys were highest for most metals in N.menziesii. On a dry weight basis, however, only the accumulation of zinc by this species was markedly different from either N.fusca or N.truncata.

When the metal concentrations in the dry leaves are considered, Table IV - 5, it was apparent that the concentrations of zinc, chromium, calcium, magnesium, manganese and potassium were higher in the November samples whereas nickel was higher in the March samples. It can also be seen that as well as zinc, iron showed higher concentrations in the dry leaves of N.menziesii than in the dry leaves of the other two Nothofagus species for both sampling surveys.

Differences in both the relative accumulations and the metal concentrations on a dry weight basis between N.truncata and N.fusca were very small.

To test the significances of the differences in the percentage ash weights and the concentrations in the leaves of zinc and iron between the three species, values of Student's "t" were calculated on the geometric means and standard deviations. These values are given in Table IV - 6 from which it can be seen that high values of t were obtained for the differences between N.menziesii and the other

TABLE IV - 5

Mean metal concentrations in the dry leaves of the three *Nothofagus* species collected in March, 1969, and November, 1969.

		p.p.m.					%			
	Survey	Ni	Cu	Zn	Cr	Fe	Ca	Mg	Mn	K
N.fusca	March	7.58	5.64	12.8	0.406	63.0	0.933	0.231	0.0549	0.304
	Nov.	4.19	4.89	15.1	0.505	53.3	0.975	0.257	0.0715	0.549
N.truncata	March	7.45	4.40	11.0	0.363	50.4	1.03	0.203	0.0484	0.271
	Nov.	5.76	4.42	13.7	0.540	57.6	1.15	0.224	0.0681	0.481
N.Menziesii	March	5.91	5.20	19.3	0.407	80.5	0.623	0.155	0.0500	0.263
	Nov.	5.84	7.36	22.4	0.554	78.0	0.780	0.203	0.0588	0.461

TABLE IV - 6

Values of Student's "t" for the differences in the percentage ash weights and the dry leaf concentrations of zinc and iron, between the three *Nothofagus* species.

	N.menziesii : N.fusca		N.menziesii : N.truncata		N.fusca : N.truncata	
	March	Nov.	March	Nov.	March	Nov.
Percentage ash weights	10.98^{++}	9.90^{++}	10.66^{++}	10.15^{++}	0.134	1.55
Zinc	12.71^{++}	5.66^{++}	10.70^{++}	7.04^{++}	1.55	2.60^{+}
Iron	7.56^{++}	6.51^{++}	9.00^{++}	6.45^{++}	1.78	2.13

Levels of significance: ++ < 0.001

\+ < 0.01

two species. These were in contrast to the much lower values obtained between N.fusca and N.truncata.

3. Discussion.

The results of both sampling surveys showed that the three species of the Nothofagus genus could be divided into two groups, with N.menziesii in one and N.fusca and N.truncata in the other, on the basis of the metal contents in their leaf ash, their percentage ash weights and the zinc and iron concentrations in their dry leaves. It was also shown that the latter three variables were significantly different between the two groups.

In SECTION III it was observed that as the leaf area increased from the Nothofagus genus through W.racemosa to Q.acutifolia, the mean percentage ash in the dry leaves also increased in the order 4.05, 4.65, 5.05. From the results presented in the present study it was apparent that the mean percentage ash in the smaller leaves of N.menziesii (see Plate II), was lower i.e. 3.23 - 3.44 than in the larger leaves of the other Nothofagus species i.e. 4.90 - 5.24. It seemed therefore, that there was a relationship between the leaf area and the percentage inorganic material in the leaf.

In SECTION III it was suggested that potassium and manganese bore some relationship to leaf area and when the relative accumulations of these metals in the dry leaves were compared to the leaf length a good relationship was evident (Table IV - 7). The precise functions of potassium and manganese in the leaf tissues have not been clearly

TABLE IV - 7

Leaf length in relation to the mean relative accumulations of potassium and manganese in the dry leaves.+

Species	Leaf length (cm)++	K(%)	Mn(%)
N.menziesii	0.8 - 1.2	0.602	0.435
N.fusca	2.5 - 4.0	0.753	0.522
N.truncata	2.5 - 4.0	0.759	0.565
W.racemosa	2.5 - 10.0	0.836	0.525
Q.acutifolia	6.0 - 16.0	2.23	0.770

\+ Values for the Nothofagus species are the means of all samples from both sampling programmes. (Soils, -120 mesh).

++ Data from Poole and Adams (1963).

established but the present results may be related to an increasing proportion of chloroplasts in the leaf with increasing leaf area.

The differences in the zinc and iron contents of the dry leaves between N.menziesii on the one hand and N.fusca and N.truncata on the other, may be of genetic origin. Cockayne (1926) and Cockayne and Atkinson (1926) indicated that N.fusca and N.truncata must be closely related genetically because they undergo hybridisation.whereas N.menziesii would not be so closely related for they found no evidence of hybridisation between the latter species and any other. Cockayne and Atkinson (1926) also showed that the other two Nothofagus species N. solandri and N.cliffortioides can form hybrid swarms with N.truncata and N.fusca but not apparently with N.menziesii. It would be of considerable interest to know how the metal concentrations in the dry leaves of N.solandri and N.cliffortioides compare with those in the dry leaves of N.fusca and N.truncata. If, as the present results tend to indicate, N.cliffortioides and N.solandri have relative accumulations of nickel similar to those of N.fusca and N.truncata, then the usefulness of biogeochemical prospecting for this metal could be extended above the maximum altitude level of about 3,500 feet for the latter two species, to over 4,000 feet where N.cliffortioides predominates (Cockayne, 1926). This is a possibility which could be considered in future extensions of biogeochemical prospecting using the Nothofagus genus.

D. THE RELATIONSHIPS BETWEEN THE METAL CONCENTRATIONS IN THE LEAVES OF THE NOTHOFAGUS SPECIES AND THE METAL CONCENTRATIONS IN THE SOIL.

1. Introduction.

In the preliminary studies (SECTION III) it was assumed that the coarse fraction (-20 mesh) of the soil was a good representation of the plant substrate. Before further, more detailed investigations were made, however, it was necessary to find how justified the initial assumption was.

2. Preparation of soil fractions.

(a) Methods.

Four soil fractions were considered, and these were prepared as follows: Three portions of the -20 mesh fraction were taken, two of which were further sieved to yield a -120 mesh fraction and a -400 mesh fraction. The total metal concentrations in all three fractions were determined. In addition, the extractable metal concentrations in the -20 mesh fraction was determined.

It is well known that the apparently simple process of sieving is in fact quite complicated and the statistics of the process have been discussed by Griffiths (1967). With respect to the present study, the fractionation of resistant from non-resistant minerals as a function of sieving time was important. This effect, as well as the possibility of contamination from the brass sieve used, was studied briefly for the -400 mesh fraction.

(b) The extents of contamination and fractionation during sieving.

(i) Methods.

The following experiment was carried out: A sample of soil was passed through the -400 mesh sieve four times. At each stage the sieve was shaken for one minute and the material which did not pass through the sieve was discarded. A small portion (0.200gm) was taken of the material which passed through the sieve at each stage. Each fraction was analysed and the results are shown in Table IV - 8.

(ii) Results.

The results of this experiment showed that of the metals analysed, nickel, zinc, manganese and potassium showed no change in concentration with progressive sievings. On the other hand copper, chromium, calcium and magnesium all showed increases to varying extents. The increases were expressed as percentages of the concentrations in the first fraction and these values are also shown in Table IV - 8. The greatest increase observed was for copper.

(iii) Discussion.

Since the composition of brass is approximately 66% copper and 30% zinc and if the increase in the copper concentration observed with successive sievings was

TABLE IV - 8

Analyses of a soil fraction at each stage of four successive passings through a 400 mesh brass sieve.

No. of times	p.p.m.				%			
sieved	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
1.	170	17.5	85	825	4.60	4.30	0.100	0.55
2.	170	18.8	85	830	4.60	4.35	0.095	0.52
3.	170	21.0	85	855	4.80	4.50	0.100	0.55
4.	170	22.5	85	880	4.90	4.70	0.095	0.55
% increase between the 1st & 4th sievings.	0.0	28.6	0.0	6.7	6.5	9.3	0.0	0.0

due to contamination, then a small increase in zinc would also have been observed. No such increase was, in fact, apparent and in view of this it was considered that the observed results were probably due to mineral fractionation.

Both primary silicate (unweathered) and secondary minerals (clays and hydrous oxides) were present in the soil. The more mechanically-resistant primary silicate minerals of the Complex contained only small amounts of copper whereas the secondary minerals contained a considerable amount of exchangeable copper derived from the chemical weathering of small copper sulphide deposits. The continuous removal of primary minerals during the sieving process resulted in an increasing proportion of secondary minerals in the fraction passing through the sieve and hence an increase in the copper content of this fraction. Further comment on this effect is made in Part 3 of this section.

(c) Evaluation of different soil extractants.

(i) Methods.

Preliminary experiments showed that extraction of the soils with water removed very small amounts of the trace metals from most samples and in the presence of the organic matter also extracted these small amounts could not be accurately determined.

Approximately 30, -20 mesh soil samples were extracted with the following solutions: 1 M ammonium acetate, 0.5 M acetic acid, 0.05 M disodium ethylenediaminetetraacetate and 1 M hydrochloric acid. Four gm of soil were shaken with 10.0 ml of extractant for 6 hours. The samples were centrifuged and the supernatant analysed directly for copper and nickel.

The approximate mean concentrations extracted by these extractants were (nickel concentration first figure, copper concentration second figure); hydrochloric acid, 10p.p.m., 13p.p.m.; disodium E.D.T.A, 4p.p.m, 6p.p.m.; acetic acid, 1p.p.m., 0.4p.p.m., ammonium acetate, 0.3p.p.m., 0.2p.p.m. The dilution factor used (weight of solution: weight of soil) was 2.5 which meant that the concentrations in solution were in the range 5p.p.m. to less than 0.1p.p.m. Levels below 1p.p.m. could not be determined accurately in the solutions due to interference, apparently from organic material. Since no further purification of the extracts was practical, the choice remained between hydrochloric acid and disodium E.D.T.A. There appeared to be little difference between the results obtained for these two extractants when compared to the total concentrations in the plants and the soils. 1 M hydrochloric acid was therefore chosen because of the higher concentrations extracted with this solution. It was noticed that the extraction of most metals by hydrochloric acid was time-dependent and a further experiment was performed to investigate this.

(d) Time dependence of the metal concentrations extracted from soil by 1 M hydrochloric acid.

(i) Methods.

Five soil samples were each

divided into six parts and each part was extracted for a different period of time. In each case 10.0ml of 1 M hydrochloric acid was added to 4.0gm of soil in a 20.0ml glass phial. This phial was rotated about an axis through its ends with the axis horizontal. The speed of rotation was 15 revolutions per minute. Samples were removed at intervals of 0.75, 1.5, 3, 6, 12 and 24 hours, centrifuged and filtered where necessary to remove floating organic matter. The solutions were analysed without further dilution for nickel, copper, zinc and chromium and after a dilution of 50X with 0.8% $Sr(NO_3)_2$, for calcium, magnesium and potassium.

(ii) Results.

The results obtained are presented in Figures IV - 2 and IV - 3. It was apparent that for nickel, copper, zinc, chromium and magnesium, the extraction was time-dependent with a minimum of six hours to achieve greater than 75% extraction. For calcium and potassium on the other hand, complete extraction was achieved in less than 45 minutes.

(iii) Discussion.

The rapid extraction of calcium and potassium seemed to indicate a single step process, whereas the extractions of the other metals implied a two step process. For each metal the first process extracted different percentages of the total amounts extracted. If, in the first 45

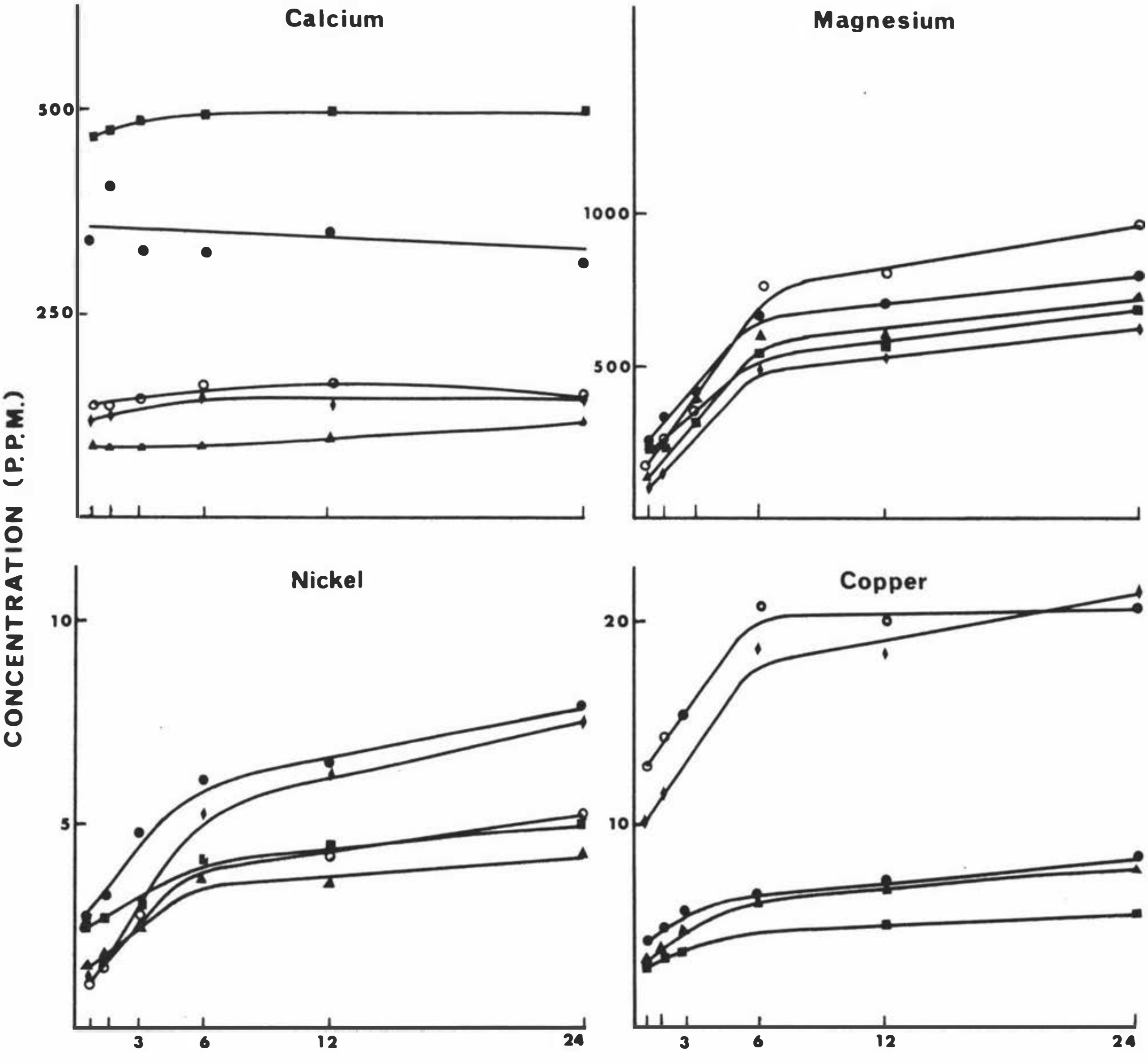


Figure IV-2. Concentrations of metals extracted from soils by cold 1M HCl as a function of time.

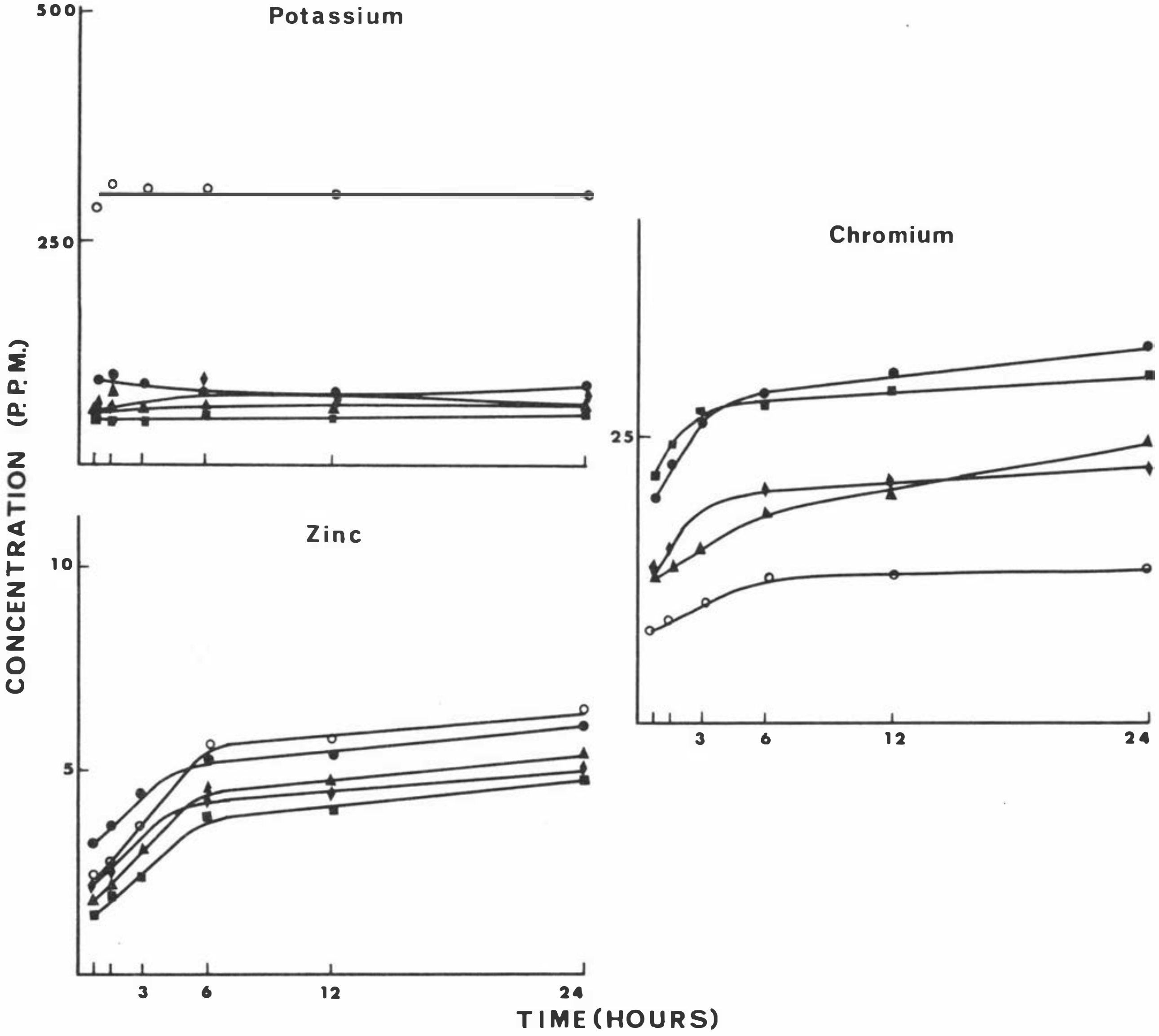


Figure IV-3. Concentrations of metals extracted from soils by cold 1M HCl as a function of time.

minutes, the first process is assumed to be dominant over the second process then the amounts extracted by this first process expressed as a percentage of the total amounts extracted were: calcium, 100%; potassium, 100%; chromium, 70%; copper, 50%; zinc, 50%; nickel, 30%; magnesium, 20%. Although these values are only approximate, they illustrate that different types of chemical bonding occur between various cations and other soil constituents.

The process effective in the first step was probably simple cation exchange. The second step possibly involved the decomposition of organic complexes and the dissolution of inorganic compounds such as hydrous iron oxides which contained occluded metals.

Although these findings have important implications with respect to the availability of cations to plants, no further investigations into this problem were undertaken in this thesis.

(e) Conclusions.

It was concluded from the above results that it was necessary to sieve all the soil samples to approximately the same extent to avoid the concentration of copper in the soil fraction. This was achieved by sieving until black mineral grains were obvious in the unsieved portion, at which stage sieving was stopped.

From the time-dependence studies of the cold hydrochloric acid extractions, it was concluded that sufficiently complete extraction was obtained after 18 hours shaking and this period was adopted as standard procedure.

3. Differences between the metal concentrations in the plants and in the various soil fractions.

(a) Results.

The mean metal concentrations in the various data sets are shown in Table IV - 9.

Within the three soil mesh fractions, the total metal concentrations showed different trends depending on the metal. Copper and zinc both showed increases in concentration while calcium and magnesium showed decreases in concentration on going from the -20 mesh fraction to the -400 mesh fraction. The greatest increase was for copper where the concentration in the -400 mesh fraction was 42% higher than the concentration in the -20 mesh fraction. For zinc, the increase was approximately 8%. The decreases for calcium and magnesium were 20% and 14% respectively.

When the hydrochloric acid-extractable metal concentrations are considered, it is apparent that the percentages of the total concentrations in the -20 mesh fraction that were extractable varied from 0.1% for potassium up to 20.4% for copper.

(b) Discussion.

The most significant finding to

TABLE IV - 9

Mean metal concentrations in the leaf ash of the Nothofagus species and in the various soil fractions.

Data sets	No. of Samples	p.p.m.				%			
		Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
Nothofagus leaves	140	109	105	344	12.2	20.4	4.82	1.29	10.3
Soil (-20 mesh)	147	170	66.5	85.6	525	4.89	4.63	0.112	0.604
Soil (-120 mesh)	146	167	81.5	87.7	436	4.25	4.09	0.102	1.34
Soil (-400 mesh)	144	168	94.3	92.3	475	3.92	3.99	0.112	0.530
Soil (cold HCI -20 mesh)	146	9.30	13.6	8.44	26.1	0.0416	0.108	-	0.0101
% extractable from the -20 mesh fraction		5.8	20.4	9.9	5.0	0.85	1.5	-	0.10

emerge from these results was the increase in copper concentration which occurred with increasing proportions of fine particles in the soil fraction. As mentioned previously, it was likely that a substantial part of the copper in the soil was derived from the chemical weathering of small disseminated pockets of copper sulphides. This copper would be associated mainly with the secondary minerals such as clays and hydrous iron oxides with which the finer soil fractions were enriched. Indeed, in the -20 mesh soil fraction approximately 20% of the total copper was soluble in dilute hydrochloric acid.

This was in contrast to the nickel which in the soils and rocks was present mainly in the silicate mineral pyroxenite. The dispersion of nickel was therefore by mechanical as well as chemical weathering with the result that the secondary minerals were not enriched with nickel. The mean percentage of soluble nickel in the -20 mesh fraction of the soil was only 5.8.

These findings emphasise the difficulties in making comparisons between the absolute copper concentrations in plants and soils without very specific knowledge of the soil fraction concerned. It is shown in a later part of this section, however, that although the concentrations of copper varied between different soil fractions, the distributions of copper over the grid area were the same for each fraction.

4. Correlation coefficients between the metal concentrations in the plants and in the various soil fractions.

(a) Results.

The correlation coefficients obtained are shown in Table V - 10.

Highly significant (<0.001) correlation coefficients were observed between the nickel concentrations in the leaf ash and the concentration of this metal in the soil fraction. Coefficients significant at the 0.01 level were observed in the case of copper. All other correlations between the plant and soil data sets were insignificant.

Little difference was apparent between the correlations on an ash weight basis and those obtained on a dry weight basis for nickel whereas for copper the correlations on an ash weight basis were slightly more significant than those obtained on a dry weight basis.

(b) Discussion.

It was apparent from the results obtained that all soil fractions were equally useful as representations of the plant substrates. The -20 mesh soil fraction showed a slightly lower correlation coefficient for the case of nickel, but whether this was significantly different from those obtained for the other soil fractions was doubtful. The same conclusions were drawn for copper. These findings suggested that the original decision to use the -20 mesh soil fraction was justified.

TABLE IV - 10

Correlation coefficients between the metal concentrations in the leaves and the metal concentrations in the various soil fractions.

Soil fraction	No. of Samples	Ni	Cu	Zn	Cr	Ca	Mg	Mn	K
Ash									
-20 mesh	140	0.504^{++}	0.307^{+}	0.216	0.066	-0.041	0.097	-0.119	0.065
-120 mesh	139	0.546^{++}	0.296^{+}	0.159	0.069	-0.043	0.069	-0.099	0.092
-400 mesh	138	0.524^{++}	0.269^{+}	0.113	0.035	-0.015	0.110	-0.171	-0.058
Cold HCl extract (-20 mesh)	139	0.545^{++}	0.306^{+}	0.050	-0.022	-0.134	0.173	-	-0.075
Dry									
-20 mesh	140	0.533^{++}	0.263^{+}	0.175	0.150	-0.013	0.122	-0.136	0.026
-120 mesh	139	0.556^{++}	0.261^{+}	0.082	0.143	-0.065	0.109	-0.176	0.194
-400 mesh	138	0.522^{++}	0.214	0.025	0.053	-0.018	0.129	-0.244	0.009
Cold HCl extract (-20 mesh)	139	0.519^{++}	0.239^{+}	-0.060	0.074	-0.135	0.052	-	0.199

Levels of significance ++ < 0.001

+ < 0.01

The correlation coefficients obtained were similar to those obtained for the Nothofagus genus in the preliminary survey (SECTION III) and supported the decision made in that section to chose this genus for further study.

The slight differences observed between the correlation coefficients based on an ash weight and those based on a dry weight basis supported the preferential use of concentrations based on ash weights.

E. THE USE OF TREND ANALYSIS TO COMPARE AND INTERPRET BIOGEOCHEMICAL AND GEOCHEMICAL DATA.

1. Introduction.

In previous discussions, plant soil relationships for various metal concentrations were evaluated by computing correlation coefficients. These coefficients gave some idea of the "strength" of the relationships but no idea of the processes involved.

Any attempt to interpret a data set in terms of the processes involved is usually limited by the experimental errors inherent in the data. This is particularly so with the type of data considered in this thesis (Miesch, 1964). The usual way of overcoming this problem is to utilise a statistical technique to separate the data into components, some attributable to meaningful processes and others to experimental error.

There are two statistical procedures which have become popular in recent years for dealing with this type of data, factor analysis and trend analysis.

(a) Factor analysis.

Factor analysis is a term applied to a statistical procedure which is used to separate a set of variables into groups (usually called "factors"), where each factor is a result of a particular process. Any data set, containing more than one variable, will be a function of a number of different processes. For example, in the case of the metal concentrations in the leaves of the *Nothofagus* genus, these processes are those depicted in the biogeochemical cycle discussed in SECTION I. To illustrate the type of information factor analysis could give, a hypothetical result which might be produced by factor analysis of the *Nothofagus* data set is discussed below.

If this data set was subjected to factor analysis the mathematical procedure would possibly derive four main factors as follows:

(1) nickel and copper

(2) copper, zinc, calcium, magnesium, manganese, iron and potassium

(3) zinc and iron

(4) chromium.

For each factor, the relative weightings of each variable in the factor would be calculated as well as the amount of the variance in the original data set explained by each of the four factors. Once the factors have been derived, it remains for them to be interpreted in terms of known processes.

In view of the previous results obtained in this thesis, Factor (1) would be interpreted as due to mineralisation in the soil, Factor (2) to the essential nature of the metals to the plant's nutrition and Factor (3) to the differences between *N. menziesii* and the other two *Nothofagus* species. More investigations would be necessary to interpret Factor (4). It is, however, very likely that more than four factors would be evolved to explain all the variance in the original data but some of the factors would explain only small amounts of the variance and would therefore be ignored.

The above example, although entirely hypothetical, illustrates the type of information factor analysis could give when applied to the study of metals in plants. The general theory of factor analysis has been discussed by Cattell (1965) and applications to geochemical problems have been described by Cameron (1968; 1969) and Garrett (1967).

Although the above example illustrated a possible application of factor analysis to the present study, the method had one disadvantage; it could not give a suitable basis for comparison between the metal concentrations in the plants and the metal concentrations in the soil. Since this comparison was one of the prime objectives of this study, factor analysis was not used. Trend analysis, however, as well as being a very good method for making comparisons between different sets of map data, can also give results which may be interpreted in terms of the processes involved, and can therefore replace to a small extent the functions of factor analysis.

(b) Trend analysis.

The purpose of this technique is to separate observed map data into various components, each of which may be more easily interpreted than the raw data in terms of environmental influences.

This technique involves the fitting of surfaces-of-best-fit by regression analysis procedures to the observed data. These surfaces are given by a regression equation from which an estimate of the value of the variable measured(called the "dependent" variable) may be made for each point on the area sampled. These estimated values lie on the surface-of-best-fit. The regression equation has the form:

$$T = K + K_1 L_1 \ldots\ldots + K_n L_n + \ldots\ldots$$

where T = the estimated value of the variable at point

(X,Y) on the sampling area.

K = regression constant

$K_1 \ldots\ldots K_n$ = regression coefficients

$L_1 \ldots\ldots L_n$ = terms in the co-ordinates X and Y.

These terms are the "independent" variables and may be polynomial or non-polynomial.

To obtain the components of the data set, the behaviour (trend) of the environmental processes (regional processes) which act over the major part of the area are assumed to be given by the surface-of-best-fit, and the deviations of the observed data from this surface contain the error component of the data. Thus, in theory, the method enables the behaviour of the regional processes to be examined free of experimental error. In practice, this situation is almost achieved but the surface can be influenced by other effects which will be explained below with reference to geochemical data.

The application of trend analysis to geological and geochemical map data has been discussed and illustrated by a number of workers over the last two decades (Agterberg, 1964; Cameron, 1968; Connor and Miesch, 1964: Krumbein, 1956; 1959; 1966; Miesch and Connor, 1968; Nichol *et al.*, 1969).

In the case of geochemical data, the results may be considered as having three main components:

(a) Regional trends due to large-scale geological processes such as changes in the nature of the bedrock which affect the major part of the sampling area.

(b) Local deviations from the regional trend. Deviations of this kind extend over more than one sample site and generally arise from small-scale geological anomalies such as mineralisation.

(c) Random variation over the sampling area, usually called "noise" which is caused by sampling and analytical error (Miesch,1964; Miesch and Connor, 1968).

To obtain these three components from a trend analysis procedure, the surface-of-best-fit is considered to be the regional component. The local and noise components are contained in the deviations of the observed data from the regional trend. These deviations are usually called "residuals" and the latter two components may be separated by observing the sign of adjacent residuals. Where a group of adjacent residuals all have the same sign they are said to be "autocorrelated" (Agterberg, 1966; Connor and Miesch, 1964) and in this case positive autocorrelated residuals are reflecting regions of anomalous local influence. If the dependent variable is a metal concentration in the soil, then these anomalous features may be mineralised zones. Residuals whose signs vary in a random fashion, i.e. are not autocorrelated, contain the noise component of the data.

Although in most prospecting applications, it is the autocorrelated positive residuals which are of the most interest, the actual trend surface may also be useful to indicate the regular behaviour of the data over the area concerned.

The method of trend analysis used in this thesis was that of Miesch and Connor (1968) who suggested a stepwise multiple regression procedure using non-polynomial as well as polynomial terms to the fifth degree. Their method was based mainly on the method of Efroymson (1960) and enabled independent terms to be individually added to or deleted from the regression equation depending on the degree of improvement they made to the equation. The level of significance for the degree of improvement was specified with the input data.

For use in this thesis a programme based on their method was written by the author in FORTRAN II - D and a discussion of this programme is given in the appendix.

2. Data distributions.

Since regression analysis is based on the assumptions of normal law, it is necessary that the distribution of the dependent variable be normal or at least symmetrical (Garrett, 1968; Miesch, 1964; Middleton, 1963).

It was found that almost all the data sets used in this thesis were positively skewed and because of this, a base ten logarithmic transformation was made standard procedure prior to the calculation of correlation coefficients.

For multiple regression analysis, however, the distributions of the data were more critical and histograms were constructed for both the raw data and the logarithmic data sets. Inspection of these histograms for the nickel and copper concentrations in the leaf ash of the Nothofagus species and the -20 mesh soil fraction showed that the raw data in all cases were positively skewed. By transformation to base ten logarithms this skewness was reduced. The resulting distributions were approximately symmetrical and showed only minor kurtosis. Since the other soil fractions showed similar distributions for their copper and nickel concentrations to the -20 mesh fraction, they were also transformed to base ten logarithms

3. The application of trend analysis to the present study.

(a) Introduction.

The two primary objectives of this part of the thesis are summarised as follows:

(1) To compare the plant and soil concentrations of both nickel and copper on the basis of regional trends and local deviations, and

(2) to interpret the trends and deviations in terms of biogeochemical processes.

The first of these aims was achieved by computing the surfaces-of-best-fit at the 0.05 level of significance for each data set and comparing these surfaces and the positive autocorrelated residuals while neglecting the remaining residuals which contained the error components.

(b) Results.

Iso-concentration contours were plotted to encompass the top 30% of each data set. These contours for both the raw data and the positive autocorrelated residuals as well as the trend surfaces are shown in Figure IV - 4 for nickel and Figure IV - 5 for copper. The trend equations obtained in each case are shown in Table IV - 11.

(i) Nickel.

The results shown in Table IV -11 and Figure IV - 4B, indicated that for nickel, the -120 mesh and the -400 mesh soil fractions showed slightly greater similarity to the plant data than did either the -20 mesh fraction or the cold hydrochloric acid fraction.

If the trend of the leaf ash data is compared to the trend for the -120 mesh soil fraction data, it can be seen that as the nickel concentration in the soil increased, the concentration in the plant also increased but at a slower rate. This indicated that the actual accumulation of nickel by the plant was decreasing as the nickel concentration in the soil was increasing.

The autocorrelated positive residuals for each set are contoured in Figure IV - 4C. It can be seen from this diagram that the residuals for each set show a greater similarity with each other than do the raw data contours. (Figure IV - 4A).

Figure IV - 4 Trend surfaces and iso-concentration contours of both the observed data and the positive autocorrelated residuals for nickel in the plants and soils.

1 Observed data
2 Trend surfaces
3 Positive autocorrelated residuals

A *Nothofagus* spp. leaf ash
B Soil, -20 mesh fraction
C Soil, -120 mesh fraction
D Soil, -400 mesh fraction
E Soil, cold HCl extract

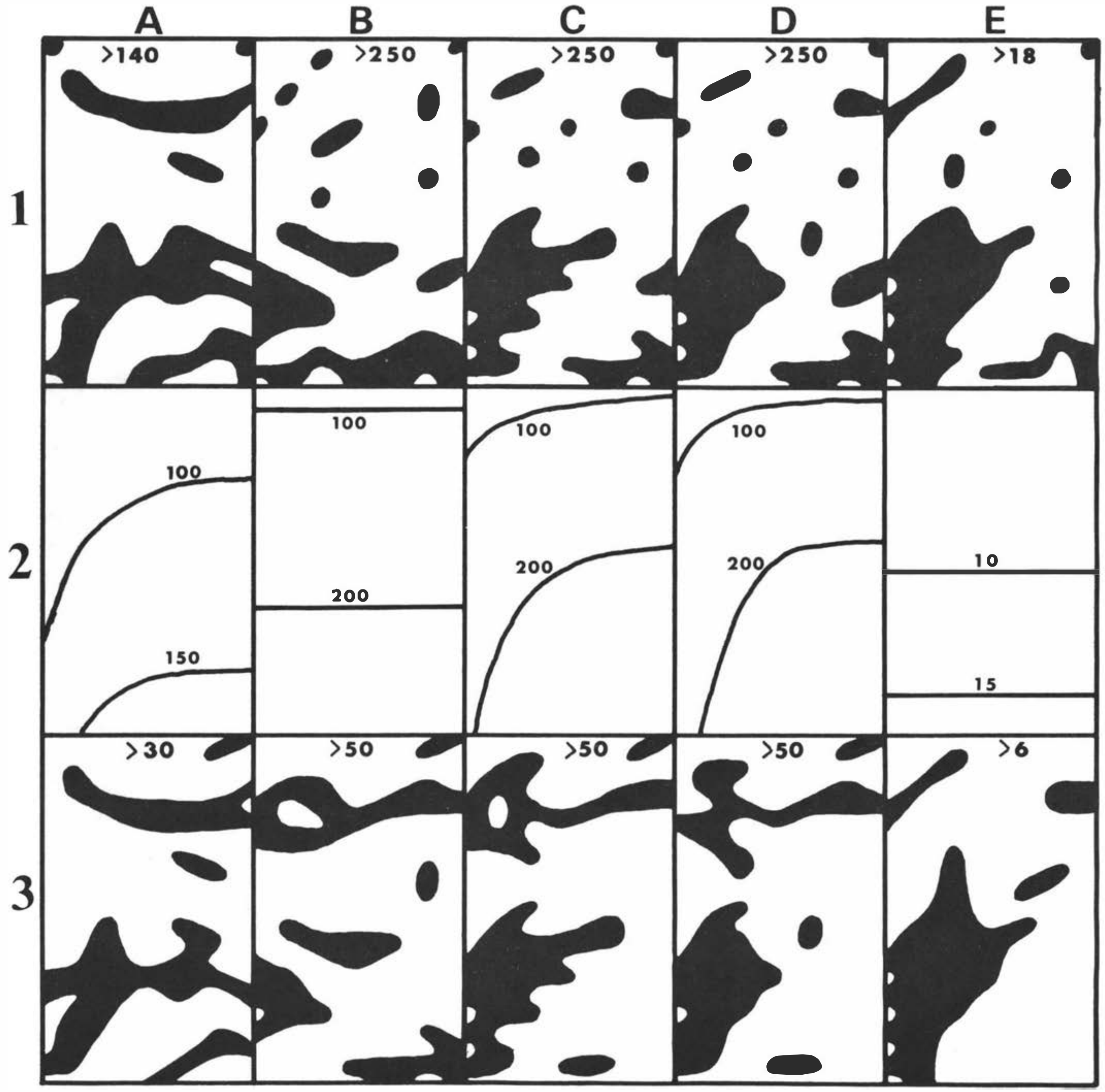

A
B
C
D
E
1
2
3
>140
>250
>250
>250
>18
100
200
150
100
200
100
200
100
200
10
15
>30
>50
>50
>50
>6
N

Figure IV-4

Figure IV - 5 Trend surfaces and iso-concentration contours of both the observed data and the positive autocorrelated residuals for copper in the plants and soils.

1 Observed data
2 Trend surfaces
3 Positive autocorrelated residuals

A *Nothofagus* spp. leaf ash
B Soil, -20 mesh fraction
C Soil, -120 mesh fraction
D Soil, -400 mesh fraction
E Soil, cold HCl extract

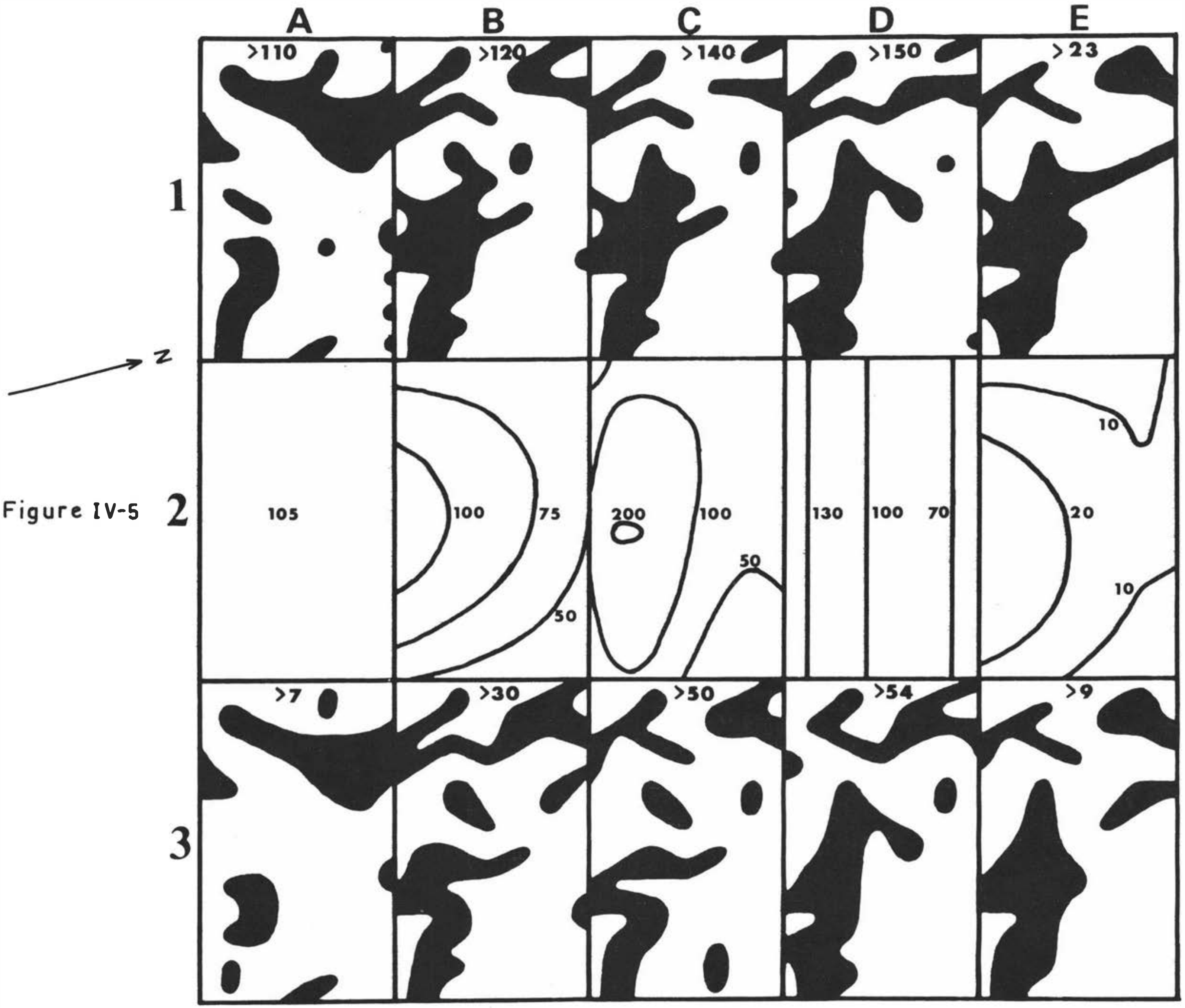


Figure IV-5

A
B
C
D
E
1
2
3
N
>110
>120
>140
>150
>23
105
100
75
50
200
100
50
130
100
70
10
20
10
>7
>30
>50
>54
>9

TABLE IV - 11

Trend equations derived at the 0.05 level of significance.

Data set	Equation	Variance Explained (%)
Nickel	Log (metal concn.) =	
Nothofagus leaf ash	$+1.890 - 0.000008X^5 + 0.166Y$	14.2
Soil -20 mesh	$+1.883 + 0.363 \text{ Log } Y$	23.0
-120 mesh	$+1.887 - 0.000010X^5 + 0.402 \text{ Log } Y$	27.8
-400 mesh	$+1.921 - 0.000013X^5 + 0.380 \text{ Log } Y$	27.6
cold HCL	$+0.720 + 0.0246\ Y$	11.2
Copper		
Nothofagus leaf ash	$+2.020$	0.0
Soil -20 mesh	$+1.717 - 0.0000025Y^4 + 0.429(\text{Log } X. \text{ Log } Y)$	16.4
-120 mesh	$+1.858 - 0.00071XY^2 + 0.0015X^3Y - 0.00016X^4Y - 0.000025X^5$	24.1
-400 mesh	$+1.800 - 0.485\ (\text{Log } Y)^2$	9.3
cold HCL	$+0.651 - 0.00000014Y^5 + 0.997(\text{Log } X. \text{ Log } Y) + 0.432X^{-1}$	17.8

(ii) Copper.

The trend equations and surfaces for the plant data (Table IV - 11 and Figure IV - 5B) showed that the leaf ash tended to contain a constant concentration of copper i.e. 105p.p.m. There was no directional component to the trend. This result was in contrast to the trend surfaces obtained for the soil fractions which all tended to a maximum at the south side of the sample grid.

The autocorrelated positive residuals for the leaf ash data reflected local deviations of the leaf ash content above the optimum value of 105p.p.m. These deviations were partly due to anomalous copper concentrations in the soil, but by visual inspection of the residual contours (Figure IV - 5C) it was apparent that the soil copper content was not the sole factor influencing these deviations.

(c) Discussion.

Some comment must be made at this point concerning the validity of the trend surfaces obtained. A measure of the "goodness-of-fit" of a trend surface is afforded by the percentage of the variance in the original data which is explained by the trend equation. This percentage variance explained by each equation is given in Table IV - 11.

Howarth (1967) has shown that linear, linear plus quadratic and linear plus quadratic plus cubic surfaces which explain less than 6.0%, 12.0% and 16.2% respectively of the total variance of the dependent variable, have a 95% chance of being derived from random data. Nichol *et al.* (1969) used trend surfaces with similar levels of explanation to compare the nickel contents of stream sediments, soils and rocks. In the resulting discussion (Parsley and Doveton, 1969; Nichol *et al.*, 1970), Nichol *et al.* pointed out that the surfaces were highly significant (>99.9%) and that they had considered the validity of them to be illustrated by the degree of correlation between the surfaces and with rolling mean analysis. Aucott (1968) and Tinkler (1969) showed that even where the explanations of surfaces fell within the limits proposed by Howarth (1967), an assessment of their validity could be made in terms of the known geology.

In the present study, some surfaces up to quintic degree were derived which showed low explanations (Table IV - 11). These surfaces were, however, considered to be valid because of the agreement shown between the different data sets. e.g. in Table IV -11 and Figures IV - 4 and IV - 5. The correlation coefficients between the data sets obtained in SECTION IV-D supported this conclusion. The results for each metal are discussed in turn.

(i) Nickel.

Although the agreement between the trend surface for the plant data and the trend surfaces for the -120 mesh and the -400 mesh soil fractions is slightly better than for the other soil fractions, the differences between the trend surfaces for all soil fractions was not great. As the -20 mesh fraction is relatively coarse, it is likely that a larger sampling error, caused by differing ratios of weathered to unweathered material would be present, with the result that the regional trend would be more difficult to detect in either the total or the cold HCl - extractable analysis of the coarse fraction.

From the similarity that was apparent between the trend surfaces for the plant and soil data, it was evident that the nickel concentration in the leaf ash was determined primarily by the concentration of this metal in the soil. It was also observed that the relative accumulation of nickel in the leaf ash decreased as the soil concentration increased. This observation may be due to a metabolic process which the plant operated to exclude nickel from its tissues as the concentration of this metal tended to become potentially toxic.

There is another possible process which may have led to the exclusion of nickel. Where there were high concentrations of nickel in the soil, the HCl-extractable nickel concentrations were also high. In addition to nickel however, all the other metals analysed in the HCl-extracts, i.e. copper, zinc, chromium, calcium, magnesium and potassium showed high concentrations in the soils containing high concentrations of extractable nickel. This was apparent even if the total concentrations of these other metals in the soils were relatively low. It was observed that these high concentrations of HCl-extractable metals occurred in the main drainage basin of the grid area and this indicated that these large extractable amounts had arisen from downhill leaching of soluble metal compounds. It therefore seemed likely that the exclusion of nickel from the leaf was due to competition between the metal ions for root absorption sites. This competition may also have arisen in the translocation processes in the plants if these metal ions move in the vascular systems as complexes. This argument implies that similar decreases in the relative accumulations of the other metals must occur also, and, in fact, this was observed (see SECTION IV - H).

The agreement shown between the autocorrelated residuals for the plant data and those for the soil data, further supported the conclusion that the nickel concentration in the plant was determined primarily by the soil concentration of nickel. These results also showed that the anomalous areas of nickel concentration as shown by the plants were a reliable guide to the areas of anomalous concentrations in the soil and in this respect biogeochemical prospecting for nickel using the Nothofagus genus is feasible.

(ii) Copper.

The correlation coefficients for the copper data (SECTION IV - D) showed that there was not a strong plant-soil relationship for this metal. From the results presented in Table IV - 11 and Figure IV - 5 the reasons for this poor relationship were apparent.

The trend towards a constant copper content in the leaf tissue suggested the existence of some metabolic process in the plant which was regulating the accumulation of this metal. It has been established that copper is essential for normal growth (Bowen,

1966) and in view of this, the present finding was not surprising. What was rather surprising was that the level maintained by the plants was so high i.e. for the *Nothofagus* genus, approximately 5p.p.m. copper on a dry weight basis. It is generally acknowledged that the specific requirement of plants for copper is very much lower than the actual amount present in their tissues and for this reason it might be assumed that the plant would exert control only over the accumulation of the required amount. The present results, however, show that the plant controls the accumulation of much more copper than it requires for its immediate use. This suggests that the extra copper does form some, as yet unknown, function or that the plant maintains "pools" of copper from which it draws its requirements.

The autocorrelated positive residuals for the plant data when compared to the autocorrelated positive residuals for the soil data indicated that although high copper concentrations in the soil tended to induce high concentrations in the leaf ash, there were some other more influential factors which caused the deviation of the copper concentrations in the ash away from the optimum level of 105p.p.m. The nature of these unknown factors was not understood but a technique of estimating their effects is presented in the next section.

(d) Conclusions.

By using trend analysis, two different sets of data, namely the metal contents of plant tissues and the metal contents of soils, were compared by separating the sampling error from each data set. The remaining data sets which contained components attributable to regional and local influences were interpreted in terms of the factors involved in the biogeochemical cycling of metals.

It was shown that the concentration of nickel in the leaf ash was determined to a large extent by the concentration of this metal in the soil, whereas for copper the concentration in the leaf ash was a function of the plant's specific requirements for this metal.

In the case of nickel it was shown that when the regional influences were removed from the plant and soil data sets, the remaining positive autocorrelated residuals for the plant data showed very good agreement with those for the soils.

It was concluded from these findings that the leaves of the Nothofagus genus were potentially useful as agents for biogeochemical prospecting for nickel in New Zealand.

F. THE IMPROVED PREDICTION OF METAL CONCENTRATIONS IN THE SOIL FROM THE METAL CONCENTRATIONS IN THE LEAF ASH.

1. Introduction.

In SECTION IV - E it was shown that the concentration of nickel in the leaf ash was determined primarily by the concentration of this metal in the soil and partly by some other factors, while for copper, the concentration in the leaf ash was regulated mainly by the plant but was also affected by some other processes. If some measure of the effects of these unknown factors on the relative accumulations could be found, then corrections could be made for them, with the result that the metal concentrations in the soil could be predicted much more accurately from the concentrations in the leaf ash.

2. Multiple regression analysis.

The most convenient way to allow for these factors was to derive a regression equation containing the influential factors as the independent variables, and the relative accumulation as the dependent variable. Multiple regression analysis has been discussed briefly as applied to trend analysis in SECTION IV - E but a further comment will be made here about the form of the equation. The equation is:

$$M = K + K_1 x_1 + K_2 x_2 + \ldots\ldots\ldots \; K_n x_n + \ldots\ldots$$

where M = relative accumulation of the metal (dependent variable)

K = regression constant

$K_1 \ldots K_n$ = regression coefficients

$x_1 \ldots x_n$ = factors influencing the accumulation of the metal (independent variables)

From this equation, at any point on the grid area, the relative accumulation could be predicted simply by measuring the factors x_1 .... x_n and carrying out the necessary computation. By dividing the concentration of the metal in the plant tissue by the relative accumulation, the concentration of the metal in the soil could be found.

3. The independent variables selected for regression analysis.

In selecting the independent variables to be tested by the regression analysis, it was borne in mind that the factors finally appearing in an equation would have to be measured during a prospecting survey either in the field or in the laboratory. This ruled out variables such as metal concentrations in the soil, pH etc. since these would involve the collection of soil samples which was to be avoided.

The independent variables chosen for the regression analysis included the concentrations of all the metals analysed in the leaf ash as well as the estimated height and measured diameter of each tree sampled. Also included were; the height at which leaves were sampled from each tree, expressed as a fraction of the total height; the slope at each sampling site measured with a protractor and plumb line and the altitude at each site measured from a survey map contoured at 50 feet intervals.

4. Distribution of the independent variables.

It was mentioned previously (SECTION IV - E) that variables used in regression analysis must have near normal distributions. However, it was shown in SECTION III - F that for nickel the relative accumulation values fell into two groups with *N.fusca* and *N.truncata* in one and *N.menziesii* in the other, and for copper the values were different for all three species. This implied bimodal and trimodal frequency distributions for the relative accumulations of nickel and copper respectively due to species differences. Since these distributions were not suitable for regression analysis, the relative accumulation values for each species and each metal were transformed to give them a geometric mean of 1.0. This removed the variance due to different species but lost none of the variance due to other biogeochemical factors.

Frequency histograms of the resulting relative accumulation values (approximately 140) were constructed for each metal. From these it was noticed that there were a few values for each metal which lay well above the bulk of the data and that these values corresponded to samples from mineralised sites. These values were removed from the sample population for the following reasons:

(1) The distributions with the high values present were multimodal and as such would have adversely affected the regression results.

(2) The main aim of this study was to evaluate the effects of factors other than mineralisation which may have influenced the results. Values which were very largely influenced by mineralisation were therefore not applicable to the regression analysis.

The above independent variables corresponding to the relative accumulation values were also checked for distribution by plotting frequency histograms. The distributions of some of the variables showed lognormal tendencies, so they, as well as the relative accumulation values, were transformed to base ten logarithms. (See Table IV - 12).

The frequency histograms for the dependent and independent variables used are shown in Appendix II.

5. Procedure.

The multiple regression analysis was carried out by the same procedure as used previously for trend analysis. The programme written for the trend analysis work was modified to accept the appropriate dependent and independent variables selected for the present study. (See Appendix IV). The derivation of the regression equation was carried out at two levels of significance, 1.0 and 0.10. At a significance level of 1.0, all the independent variables tested were inserted into the equation and at the 0.10 level of significance only those variables with a 90% probability of explaining some of the variance of the dependent variable were retained in the regression equation.

6. Results.

The equations and the percent of the variance of the relative accumulations which they explain are given in Table IV - 12.

The regression equations containing all the independent variables were found to explain 34.0% and 35.0% of the variance observed in the relative accumulations of nickel and copper respectively. However, it can be seen from the table that most of the regression coefficients contained a large error and were thus expected to be unstable with respect to slight changes in the data. On the other hand, at the 0.10 level of significance, only two independent variables were retained in the regression equation for nickel and seven for copper. These variables were those containing the least error. It should be noted that while the equations at the 0.10 level of significance explained rather less of the variance observed in the dependent variable, these equations were expected to be less susceptible to small data changes and therefore to have wider application to other data sets.

In the case of nickel, the only independent variables which were significant at the 0.10 level, were the logarithm of the nickel concentration in the leaf ash and the altitude at each sampling site.

For copper, on the other hand, seven independent variables, two physical variables and five metal concentrations, were retained in the regression equation at the 0.01 significance level.

TABLE IV - 12

Results of regression analysis.

		Copper						Nickel					
		Level of sig. 1.00			Level of sig. 0.10			Level of sig. 1.00			Level of sig.0.10		
Independent variables	Means	A	B	C	A	B	C	A	B	C	A	B	C
Log Ni concn. in plant	2.228	-0.405	0.139	-0.902	-0.447	0.165	-0.996	0.523	0.119	1.165	0.501	0.090	1.116
Log Co concn. in plant	1.793	-0.517	0.433	-0.927	-	-	-	0.008	0.266	0.014	-	-	-
Log Cu concn. in plant	1.904	0.539	0.215	1.026	0.499	0.178	0.950	0.112	0.102	0.213	-	-	-
Log Zn concn. in plant	1.943	0.185	0.311	0.359	-	-	-	0.159	0.184	0.309	-	-	-
Log Cr concn. in plant	2.638	0.929	0.319	2.451	0.301	0.242	2.113	0.205	0.193	0.541	-	-	-
Log Fe concn. in plant	3.091	-0.127	0.288	-0.393	-	-	-	-0.115	0.160	0.355	-	-	-
Ca concn. in plant	4.431	-0.057	0.045	-0.253	-0.056	0.040	-0.243	0.028	0,027	0,124	-	-	-
Log Mg concn. in plant	0.617	0.997	0.444	0.615	1.019	0.386	0.629	-0.277	0.260	-0.140	-	-	-
Log Mn concn. in plant	0.098	0.107	0.233	0.010	-	-	-	0.061	0.139	0.006	-	-	-
K concn. in plant	1.447	-0.014	0.016	-0.020	-	-	-	0.003	0.009	0.004	-	-	-
Ash as % of dry weight	4.895	-0.002	0.047	0.010	-	-	-	-0.001	0.027	-0.005	-	-	-
Slope of terrain	33.51	-0.005	0.004	-0.167	-0.006	0.004	-0.201	0.001	0.003	0.034	-	-	--
Altitude of sample site	210.0⁺	0.0002	0.002	0.042	-	-	-	0.003	0.001	0.630	0.003	0.001	0.630
Log of tree height	1.257	0.052	0.321	0.065	-	-	-	0.141	0.200	0.177	-	-	-
Log of tree diameter	0.255	0.204	0.255	0.052	0.219	0.097	0.056	-0.070	0.155	-0.018	-	-	-
Sampling height as fraction of tree ht.	0.563	-0.005	0.176	-0.003	-	-	-	0.109	0.106	0.062	-	-	-
Value of regression constant		-1.678			-2.332			-2.627			-1.701		
% of variance in relative accumulation explained by regression equation.		35.0			32.5			34.0			26.5		

A - value of regression coefficient
B - error in regression coefficient
C - Mean of independent variable multiplied by regression coefficient
+ - altitude reduced by factor of 10 to aid computation.

7. Discussion.

(a) Nickel.

Since the total variance of the relative accumulation values is a function of the variances of the concentrations of nickel in both the leaf ash and the soils, it was expected that the concentration of nickel in the leaf ash would appear as a term in the regression equation. It was noticed in the trend analysis results (SECTION IV - E) that the relative accumulation of nickel tended to decrease as the concentration of this metal in the soil increased. The trend equations also showed that the higher concentrations of nickel in the soil were in the lower altitude region of the sampling grid. This was probably due to downhill leaching of soluble nickel. In the regression equation derived in this section for nickel, the altitude term was inserted to allow for the decrease in the accumulation of this metal with increasing nickel concentration in the soil. This equation was considered likely to have general application to the area in the vicinity of the study region since in any steep terrain a similar drainage basin accumulation of nickel would be expected, with the resulting relationship between the accumulation of nickel by the plant and the altitude.

(b) Copper.

Of the seven independent variables retained in the regression equation for copper, the most influential were the concentrations of chromium, nickel and copper in the leaf ash. (see column C in Table IV - 12). The relative accumulation increased with increasing concentrations of chromium and copper and decreased with increased concentrations of nickel. The presence of copper in the regression equation was expected as explained above for nickel. The fact that the relative accumulation increased with increasing chromium concentration in the leaf seemed to indicate some type of mutual stimulation of accumulation between copper and chromium, while in the case of nickel the effect seemed to be antagonistic.

The other independent variables retained in the regression equation were all less important. For the other metals, calcium and magnesium, the reasons for their effects were probably very complex and associated with the effects of nickel and chromium.

The slope of the terrain made a significant contribution with the relative accumulation increasing with decreasing slope. This may be interpreted as a function of drainage with a greater accumulation in areas of poor drainage. This effect was also observed by Mitchell et al. (1957) who noted an increasing accumulation of some metals by plants in areas of poor drainage relative to well drained soils. He attributed this observation to an increase in the "available" concentrations of these metals in poorly-drained soils.

The last and least significant variable was the tree diameter; with the relative accumulation increasing with increasing tree size. However, the contribution this term made to the regression equation was negligible.

To evaluate the usefulness of the equations derived at the 0.10 level of significance, they were used to predict the soil concentrations of copper and nickel from the concentrations of these metals in the leaf ash and the values of the influential factors.

Table IV - 13 lists the correlation coefficients of the observed concentrations in the soil with both the plant data and the predicted soil data. For both metals, the correlations are higher for the predicted soil data than for the plant data. For nickel, the improvement is not great because the plant data already correlated well with the soil data but for copper the improvement due to the regression equation is considerable.

Graphs of the plant versus the observed soil and the predicted soil versus the observed soil data sets are presented in Figure IV - 6. The same general improvement can be seen.

The predicted concentrations in the soil were subjected to trend analysis and the trend surfaces derived at the 0.05 level of significance are compared with those for the plant data and for the observed soil data. (Figure IV - 7).

In the case of nickel, it can be seen that the trend surface of the predicted soil concentrations was a slightly different shape to either the surface for the plant data, or the surface for the observed soil data. However, the actual concentration contours of the surface for the predicted data corresponded very closely in position to those of the surface for the observed soil data with the exception of the lower concentration regions. From the observed

Figure IV - 6 Concentrations in the Nothofagus spp. leaf ash and predicted concentrations in the soil (0.10 level of significance) plotted against the observed concentrations in the soil for nickel and copper.

A Nickel in the plant versus observed nickel in the soil.

B Predicted nickel in the soil versus observed nickel in the soil.

C Copper in the plant versus observed copper in the soil.

D Predicted copper in the soil versus observed copper in the soil.

N.B. Correlation coefficients (r) are shown on the graphs.

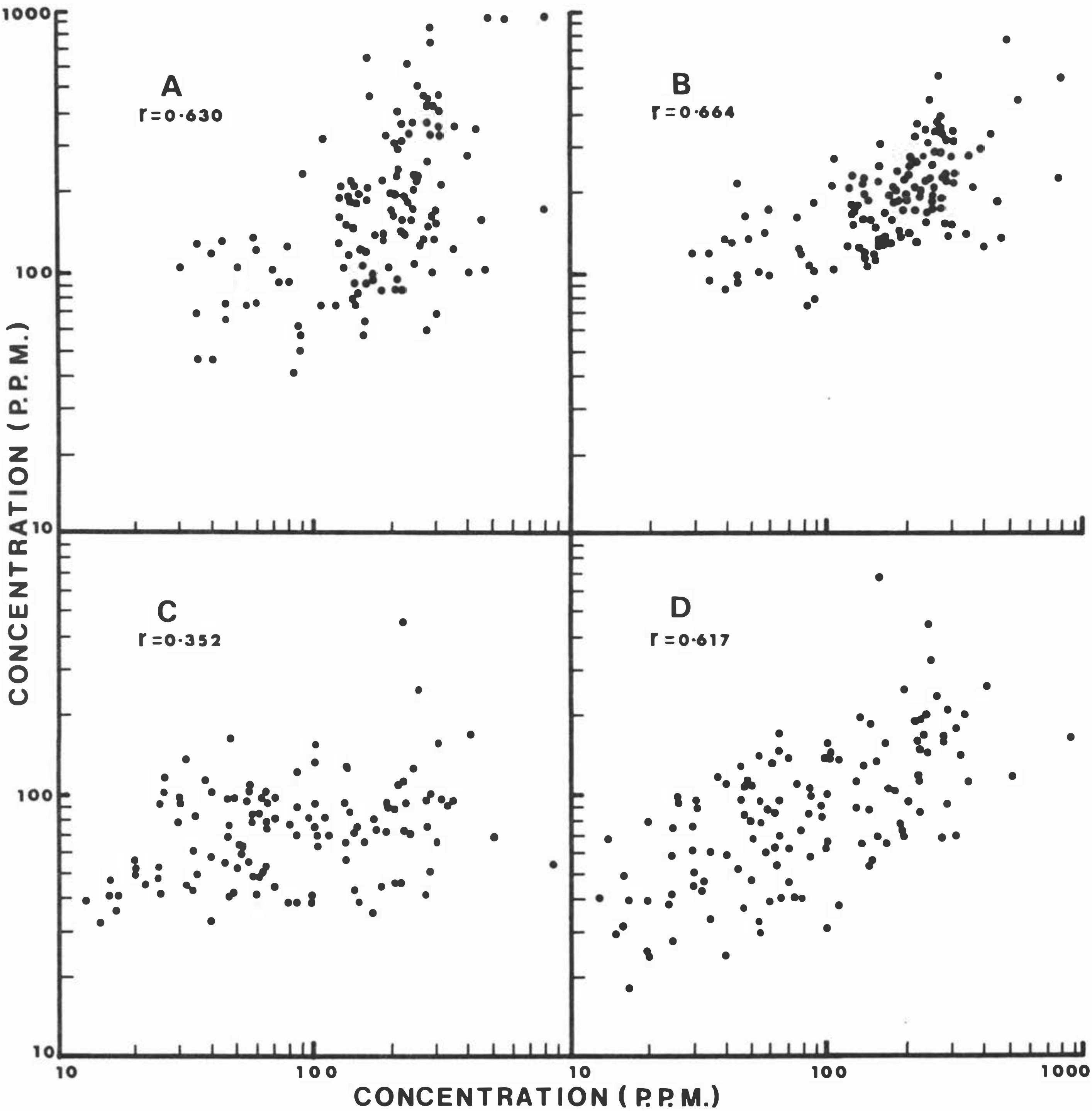

A
r = 0·630
B
r = 0·664
C
r = 0·352
D
r = 0·617
1000
100
10
10
100
10
10
100
1000
CONCENTRATION (P.P. M.)
CONCENTRATION (P.P. M.)

Figure IV-6.

Figure IV - 7 Trend surfaces (0.05 level of significance) of the observed and predicted data.

A Nickel concentrations in the *Nothofagus* spp. leaf ash.

B Predicted nickel concentrations in the soil.

C Observed nickel concentrations in the soil.

D Copper concentrations in the *Nothofagus* spp. leaf ash.

E Predicted copper concentrations in the soil.

F Observed copper concentrations in the soil.

N.B. Concentrations in p.p.m.

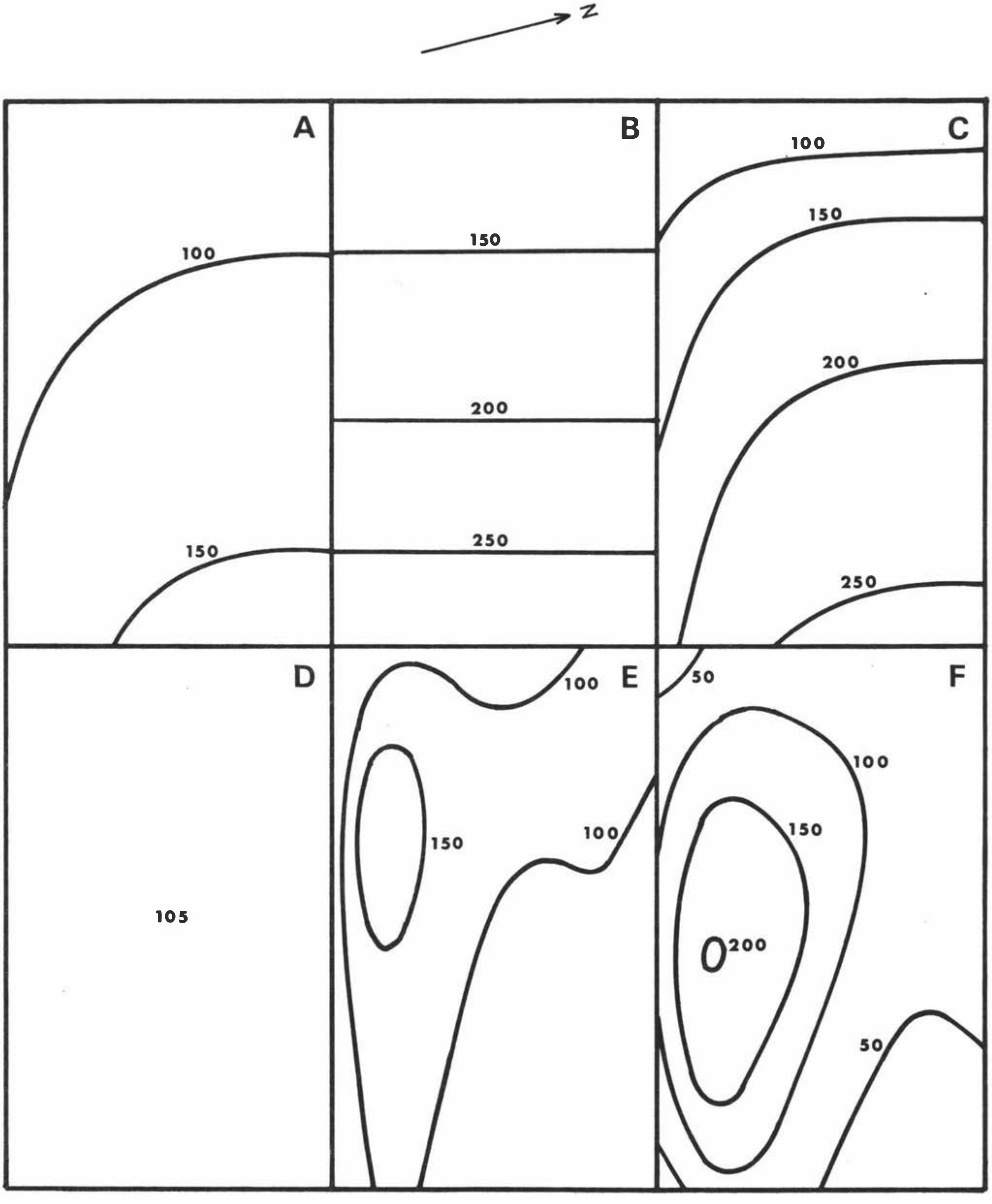

N
A
100
150
B
150
200
250
C
100
150
200
250
D
105
E
100
150
100
F
50
100
150
200
50

Figure IV-7.

TABLE IV - 13

Correlation coefficients for nickel and copper between the observed concentrations in the soil and both the predicted concentrations in the soil and the concentrations in the leaf ash.

First variable	Second variable	No.of samples	Correlation Coefficients	
Copper in leaf ash	Copper in soils (observed)	124	0.352	
Nickel in leaf ash	Nickel in soils (observed)	121	0.630	
			Signif.0.10	Signif.1.00
Copper in soils (predicted)	Copper in soils (observed)	124	0.617	0.635
Nickel in soils (predicted)	Nickel in soils (observed)	121	0.664	0.705

soil trend it can be seen that there was a rapid increase in nickel concentration over a relatively short distance in the vicinity of the sediment/basic rock contact (SECTION III - A) at the west side of the grid. The plant analyses did not detect this contact with the same degree of precision, and as a result the contact was not apparent in the predicted data. However, the general agreement of the predicted with the observed soil data was very good.

The copper concentration in the leaf ash was found to have no directional trend but a constant content of copper over the whole area. On the other hand, the trend surface for the predicted concentrations of copper in the soil showed considerable similarity to the trend surface for the observed concentrations of this metal in the soil.

Although differences were apparent between the latter two surfaces, the essential features of both were the same.

8. Conclusions.

It was apparent from the results obtained for copper that the interactions between elements in plants are extremely complicated. It is also quite probable that most of the less significant factors in the equations would be absent or changed in an area of different geology or topography. However within the same geological-ecological province only the independent variables which made small contributions to the variance of the relative accumulations would be expected to change, and the most important variables should.remain essentially unchanged.

The results obtained in this survey demonstrate the possibility of using multiple regression techniques in the improvement of biogeochemical data. It is often difficult to explain the variables retained in the regression equation and in all probability, different variables will have different weightings in different environments. However in most biogeochemical and geochemical surveys it is necessary to undertake an orientation survey and if regression equations are derived for this survey it is probable that they will be valid for the remainder of the prospecting area. In many exploration programmes the analytical data are stored on punched cards and with easy access to modern fast computers a multiple regression analysis of the data would be quite feasible.

In the present study only a small amount of the total variance in the observed relative accumulation values was explained by the terms inserted into the regression equations. The remaining unexplained variance was due to the influences of soil factors and to the sampling error associated with the data. By allowing the regression procedure to test other measured variables which could concievably influence the relative accumulation, an increasing amount of the variance in the relative accumulation would be explained by the regression equation. Ultimately, all the influential variables would be allowed for by the regression equation and the only variance left unexplained in the relative accumulation would be due to sampling error. The chances of achieving this situation are, however, extremely remote.

The use of this technique in plant nutrition studies could be of considerable value. The main difficulty, however, lies in the fact that the independent variables inserted into the regression equation may not be the factors which are directly influencing the dependent variable. Instead, they may be indirectly related factors such as the altitude term inserted into the regression equation for the relative accumulation of nickel. The altitude was an indirect factor related to the concentration of nickel in the soil which was the direct factor.

Even in view of this complication, multiple regression analysis has many attractive features which could be utilised with success by application to data similar to that described in this section.

G. THE EFFECTS OF HIGH CONCENTRATIONS OF NICKEL AND COPPER IN THE SOIL ON THE METAL CONCENTRATIONS IN THE LEAVES OF THE NOTHOFAGUS SPECIES.

1. Introduction.

Imbalances in the metal concentrations in plants can result from excessive concentrations of other cations, particularly transition metal cations, in the substrate. For example, iron and manganese have been shown to exhibit a relationship where high concentrations of one metal in the soil or nutrient solution reduced the concentration of the other in the plant. (DeKock, 1955; O'Sullivan, 1969; Somers and Shive, 1942; Twyman, 1951). It has also been observed that decreasing the concentration of iron in the sand culture medium increased the content of copper and decreased the ratio of calcium to potassium in both *Phleum pratense* and *Holeus lanatus* (O'Sullivan, 1969). Manganese at high concentrations in the nutrient solution was shown to reduce the accumulation of zinc, and molybdenum and cadmium were found to have a similar effect (Malavolta *et al.*, 1956; Hawf and Schmid, 1967).

The effects of high concentrations of copper in the substrate on the accumulation of other metals by plants is well known. For example, high concentrations of this metal in the soil or nutrient solution were shown to decrease the accumulation of zinc by a number of different plants (Dunne, 1956; Hawf and Schmid, 1967; Malavolta *et al.*, 1956).

Nickel has been shown to have a similar effect. As the concentration of nickel in the nutrient solution was increased the concentrations of iron and potassium decreased, while the concentrations of calcium and magnesium increased in the tops of oat and tomato plants. (Crooke and Knight, 1955)

In view of these findings, it was of interest to investigate the possibility of metal imbalances occurring in the trees growing in the sampling area, due to the high concentrations of copper and nickel in the soil.

Before studying the effects of nickel and copper in the soil on the metals in the plants, the relationships between the metals in the leaves were considered.

2. Inter-metal relationships within the leaves.

(a) Introduction.

To give maximum validity to the investigation, all the available samples of the Nothofagus genus were separated according to the three different species to give a total of 57 samples of N.truncata, 89 samples of N.fusca and 70 samples of N.menziesii. The relationships were evaluated by computing correlation coefficients.

(b) Results.

The highly significant correlation coefficients (<0.001) for each species are presented in Table IV-14.

TABLE IV - 14

Significant (<0.001) correlation coefficients (r) between metal concentrations in the leaves of the three *Nothofagus* species.

N. Truncata				N.fusca				N.menziesii			
57 samples				89 samples				70 samples			
Ash		Dry		Ash		Dry		Ash		Dry	
Variables	r	Variables	r	Variables	r	Variables	r	Variables	r	Variables	r
NixCu	0.490	NixCu	0.512	NixCu	0.342	NixMn	-0.552	NixCu	0.395	NixCu	0.381
CuxZn	0.504	CuxZn	0.518	CrxZn	0.366	CuxFe	0.435	NixMg	0.543	NixMg	0.458
CuxFe	0.544	CuxFe	0.545	CuxFe	0.467	ZnxCr	0.443	NixMn	-0.385	CuxMg	0.388
ZnxFe	0.496	ZnxCr	0.434	CrxFe	0.492	ZnxCa	0.389	ZnxMn	0.390	ZnxMn	0.479
CrxK	0.415	ZnxFe	0.490	ZnxMn	0.349	ZnxMn	0.408	CrxMg	0.518	CrxMg	0.512
		CrxFe	0.411	ZnxK	0.416	ZnxK	0.424			CaxMn	0.403
		CrxK	0.582	NixCa	-0.366	CrxFe	0.533				
		CaxMn	0.530	NixMn	-0.572	CrxK	0.415				
				CrxK	0.343	CaxMn	0.387				

The large number of highly significant correlations which were apparent for one species only, suggested that the correlation coefficients were influenced to some extent by the distributions of the data. In view of this, it was considered that only those relationships which were evident for at least two species were likely to be valid. By this criterion, there were four inter-metal relationships which were highly significant on both an ash and a dry weight basis, nickel x copper, copper x iron, chromium x potassium and manganese x zinc. In addition, there was one highly significant relationship on an ash weight basis only, manganese x nickel, and a further three on a dry weight basis only, calcium x manganese, chromium x iron and chromium x zinc.

(c) Discussion.

Since highly significant plant x soil correlation coefficients for both nickel and copper were obtained for the Nothofagus genus (SECTION IV - D) and since these two metals were highly significantly correlated in the soil (r = 0.430, -120 mesh fraction, 146 samples) the relationship observed between the nickel and copper concentrations in the plants was expected.

The inverse relationship between nickel and manganese was difficult to explain but it may be a function of the nickel concentration in the soil since the nickel in the plant was highly correlated with the nickel in the soil.

Chromium was observed to be related to potassium, iron and zinc. It had previously been noted that when a plant species contained high amounts of chromium it contained relatively low amounts of iron. However, the present results indicated that within samples of the same species, chromium was related to iron. The reasons for the other relationships with chromium were not known.

The other three relationships observed, copper x iron, zinc x manganese and calcium x manganese were also difficult to interpret but since all the metals are essential to plant nutrition the relationships may well have some physiological basis.

3. The influences of high nickel and copper concentrations in the soil on the metal concentrations in the leaves.

(a) Results.

The correlation coefficients between the nickel and copper concentrations in the soil and the metal concentrations in the leaf ash are shown in Table IV - 15.

High nickel concentrations in the soil decreased the manganese concentrations and increased the magnesium concentrations in the leaves to a significant extent. On the other hand, high copper concentrations in the soil decreased the chromium concentrations in the leaves.

TABLE IV - 15

Correlation coefficients between the metal concentrations in the leaf ash and the nickel and copper concentrations in the soil (-120 mesh).

1st correlating variable	No. of samples	2nd correlating variables						
		Concentrations in the leaf ash						
Concentrations in the soil		Zn	Cr	Fe	Ca	Mg	Mn	K
Nickel	138	-0.176	0.177	0.073	-0.093	0.313^{+}	-0.439^{++}	0.078
Copper	138	0.066	-0.316^{+}	0.104	-0.134	-0.163	-0.071	0.226

Levels of significance ++ <0.001

\+ <0.01

(b) Discussion.

The inverse relationship between the nickel in the soil and the manganese in the plant suggested that some process was involved in which high concentrations of nickel in the soil were preventing either the absorption and/or the translocation of manganese. It was unlikely that competition on the basis of ion exchange at the root surface was responsible because if this was the case, other metals would also have shown inverse relationships to the nickel in the soil. In the previous part of this section an inverse relationship was observed between the nickel in the plant and the manganese in the plant. However, because there was a highly significant plant x soil correlation for nickel it was impossible to establish whether the nickel in the soil or in the plant was causing the observed inverse relationship with manganese in the plant.

The less significant relationships between nickel and magnesium, and copper and chromium were not understood.

The results observed in this study are in contrast to those reported by other workers. Crooke and Knight (1955) reported an increase in the accumulation of manganese with increasing nutrient concentrations of nickel. Also the decrease in the accumulation of zinc as the copper concentration in the substrate was increased which had been observed by other workers, was not apparent in the present study.

Most of the previous work, however, was carried out in nutrient solution in which the diverse effects of the soil were absent and this may be one of the reasons for the differences between the reported effects and those observed in the present study.

4. The influences of high nickel and copper concentrations in the soil on the ratios of the metal concentrations in the leaves.

(a) Introduction.

It is known that the absolute concentration of a metal in a plant can vary slightly due to the age, position on the tree, etc. of the leaves sampled. However, the ratio of the concentrations of two metals in the leaves of a particular species should be approximately constant in all leaf samples, if the accumulations of both metals are regulated by the plant. Where some external influence is present the ratio could deviate from normal.

Such a concept has been used in the past for biogeochemical prospecting. Warren and Delavault (1948) suggested the use of the ratio of copper to zinc concentrations in plant tissues as a means of detecting anomalous concentrations of these two metals in the soil. These authors reported values of this ratio for a number of trees in British Columbia growing over both non-mineralised and mineralised rocks. They considered that any ratio of copper to zinc in the second year twigs greater than 0.20 to 0.25, depending on the species, could possibly be indicative of copper mineralisation. The authors pointed out that although the absolute concentrations of copper and zinc varied considerably between samples of the same species, the copper to zinc ratio was much more constant. Since that time, these workers have compiled a very comprehensive list of the mean copper and zinc concentrations, as well as the copper to zinc ratios, for a large number of different species growing over both mineralised and non-mineralised rocks. (Warren, 1962; Warren and Delavault, 1949; 1955; Warren et al., 1949; 1951; 1952; 1966)

White (1950) investigated some British Columbia copper deposits. He found that although the copper and zinc concentrations in the plants growing in the vicinity of the ore varied considerably, the ratio of copper to zinc, when greater than 0.30, was successful in finding the ore body.

Worthington (1955) used this ratio in the vicinity of a lead/zinc deposit and found that copper to zinc ratios in the plant tissues, less than 0.05, successfully delineated the ore body.

Nicolls et al, (1965) also considered the copper to zinc ratio in their study of botanical and geochemical prospecting methods in the Dugald River area, Australia. These authors apparently did not consider the ratio useful in their particular area.

In the present study all possible ratios between the metals analysed in the plants were correlated against the copper and nickel concentrations in the -120 mesh fraction of the soil.

(b) Results.

The correlation coefficients which were significant are shown in Table IV - 16. The plant x soil correlation coefficients for nickel and copper are also shown for comparison. No ratio of metal concentrations in the leaf ash gave a better correlation to the nickel content of the soil than did the nickel concentration in the leaf ash. Since the correlation coefficient between the copper concentration in the leaf ash and the concentration of this metal in the soil was 0.296, any coefficient in Table IV - 16 higher than this value indicated a potentially useful indicator of copper in the soil. By inspection of the values in the table it can be seen that there were five ratios whose correlation coefficients with copper in the soil exceeded 0.296 and a further ten significant at the 0.1 level.

(c) Discussion.

(i) Nickel.

It was observed that all the metal ratios containing nickel, except one, were significantly (<0.001) influenced by the nickel concentration in the soil. This was due to the high correlation which existed between the nickel concentration in the plant and the nickel concentration in the soil.

In addition to these relationships, it was also apparent that the ratios of manganese to potassium, to magnesium and to iron in the plant decreased as the nickel concentration in the soil increased. These relationships were almost certainly due to

TABLE IV - 16

Correlation coefficients between the inter-metal ratios in the leaf ash of the three *Nothofagus* species and the nickel and copper concentrations in the soil (-120 mesh).

Nickel concentration in the soil			Copper concentration in the soil		
Ratio	Correlation Coefficient	Signif-icance	Ratio	Correlation Coefficient	Signif-icance
Ni/Cu	0.428	++	Ni/Cu	0.428	++
Ni/Zn	0.393	++	Cu/Zn	0.191	
Ni/Cr	0.335	++	Cu/Cr	0.381	++
Ni/Fe	0.277	+	Cu/Fe	0.218	
Ni/Ca	0.440	++	Cu/Ca	0.361	++
Ni/Mg	0.344	++	Cu/Mg	0.319	+
Ni/Mn	0.430	++	Cu/Mn	0.322	++
Ni/K	0.352	++	Cu/K	0.272	+
Cu/Mn	0.308	+	Zn/Fe	0.190	
Zn/Fe	-0.300	+	Zn/Mn	0.185	
Cr/Mn	0.307	+	Cr/Fe	-0.244	
Fe/Mn	0.358	++	Fe/Ca	0.170	
Ca/Mn	0.297	+	Fe/Mn	0.187	
Mg/Mn	0.371	++	Ca/K	-0.206	
Mn/K	-0.407	++	Mn/K	-0.193	
Plant concn.			**Plant concn.**		
Ni	0.546	++	Cu	0.296	+

Levels of significance ++ < 0.001

\+ < 0.01

the fact that the manganese content of the leaves decreased as the nickel concentration in the soil increased, an observation made previously.

The other less significant relationships observed all contained manganese, except for one, and could be explained as above. One ratio was observed to contain iron and zinc, but the reason for this was not apparent.

(ii) Copper.

Four ratios of metal concentrations in the leaves were found to be influenced significantly (<0.001) by the copper concentrations in the soil; copper/nickel, copper/chromium, copper/calcium and copper/manganese. Since all these ratios contained copper as one of the metals it was apparent that the results observed were influenced by the significant plant x soil correlation for copper. However, nickel, chromium and calcium were inserted into the regression equation developed in SECTION IV - F to explain some of the variance in the relative accumulation of copper and therefore the presence of these metals in the inter-metal ratios affected by copper in the soil was not surprising. Two less significant ratios (<0.01), copper/magnesium and copper/potassium were observed and could be explained in the same way.

Since previous results obtained in this thesis for biogeochemical prospecting for copper were not encouraging, these inter-metal ratios were evaluated as indicators of anomalous concentrations of copper in the soil.

5. Ratios of the metal concentrations in the leaves as indicators of soils containing high concentrations of copper.

(a) Introduction.

It was considered that the low correlation coefficients involved between the inter-metal ratios in the plants and the copper concentrations in the soil (Table IV - 16) did not give reliable indications of the usefulness of the ratios to detect high concentrations of copper in the soil. An alternative technique was used to evaluate the usefulness of each ratio.

(b) Method of evaluation.

The procedure involved the following steps:

(1) The highest 30% (i.e. 44 samples) of the copper concentrations in the soil samples were considered to be anomalous and the sampling sites corresponding to these anomalous values were recorded.

(2) The highest 30% (or lowest, depending on the sign of the correlation coefficient with the copper in the soil) of the values for an inter-metal ratio in the plant were noted and the sampling sites corresponding to these values were recorded.

(3) The degree of overlap between the anomalous sampling

sites recorded for the soils and those recorded for the inter-metal ratio in the plants was determined and expressed as a percentage of the total number of anomalous soil sites. Perfect biogeochemical results would require 100% overlap.

Since some overlap could arise even if there was no relationship between the ratios and the copper concentration in the soil, the probability of each result arising from chance was computed. (see Appendix V for method.)

(c) Results.

The results obtained for the ratios previously shown to be influenced by the copper concentration in the soil are given in Table IV - 17. The particular value of each ratio, above or below which included 30% of the values, is given in the table.

The percentage overlap for each ratio showed that no ratio indicated more than 50% of the anomalous soil sites. There were four ratios which indicated more anomalous soil sites than the copper concentration in the plant, copper/iron, copper/calcium, copper/chromium and copper/magnesium.

The soil concentrations of copper which were predicted from the regression equation (SECTION IV - F) were subjected to the same test which showed that these values indicated 61.4% of the soil sites corresponding to anomalous concentrations of copper.

TABLE IV - 17

The evaluation of inter-metal ratios in the leaf ash for indicating anomalous concentrations of copper in the soil.

Plant Variable	Cut-off Value +	% Overlap with anomalous soil sites	Probability Level ++
Ni/Cu	$>$ 1.50	29.6	0.16
Zn/Mn	$>$ 18.18	36.4	0.083
Zn/Fe	$>$ 33.33	36.4	0.083
Ca/K	$<$ 1.76	38.6	0.051
Fe/Ca	$>$ 74.07	38.6	0.051
Cu/Zn	$>$ 0.32	38.6	0.051
Cu/K	$>$ 0.87	41.0	0.027
Fe/Mn	$>$ 1300.0	41.0	0.027
Cr/Fe	$<$ 0.008	43.0	0.012
Mn/K	$<$ 0.105	45.5	0.0043
Cu/Mn	$>$ 7.39	45.5	0.0043
Cu	$>$ 112.0	45.5	0.0048
Cu/Fe	$>$ 0.009	47.7	0.0016
Cu/Ca	$>$ 0.448	50.0	0.00049
Cu/Cr	$>$ 9.00	50.0	0.00049
Cu/Mg	$>$ 2.00	50.0	0.00049
Predicted soil copper	$>$ 110.0	61.4	0.00000012

+ Value of the plant variable above or below which the values were considered to be anomalous.

++ Probability of the observed overlap occurring by chance.

(d) Discussion.

Four ratios of the metal concentrations in the leaves were shown to be more reliable than the copper concentration in the leaves for prospecting for copper. These ratios were copper/iron, copper/calcium, copper/chromium and copper/magnesium. The copper/zinc ratio suggested by Warren and Delavault (1949) was not included.

It was shown that the concentrations of copper in the soil which were predicted from the regression equation were more reliable than the inter-metal ratios for indicating anomalous concentrations of copper in the soil. It was concluded that although the regression procedure was the most promising method for biogeochemical prospecting for copper, the inter-metal ratios gave better results than the concentration of copper in the leaves.

H. ESSENTIAL AND NON-ESSENTIAL TRACE METALS AND HOW THEIR NATURE AFFECTS BIOGEOCHEMICAL PROSPECTING RESULTS.

1. Introduction.

Probably the most important general finding from the previous parts of this thesis, was the difference that existed between the accumulations of nickel and copper by the Nothofagus species. This was shown most markedly by the trend analysis results (SECTION IV - E) and was interpreted as being due to the essential and non-essential natures of copper and nickel respectively. It seemed therefore, that if the degree of essentiality of a metal for the nutrition of the plant species to be sampled could be inferred from data collected in a preliminary biogeochemical survey, then some prediction could be made as to whether a metal would give good or poor results in a larger biogeochemical survey.

There were thus, two aims of this study:

(1) To estimate the extent to which a particular metal was essential to plant nutrition, and

(2) on the basis of this, to predict whether the particular metal/plant system was likely to give successful results in a biogeochemical prospecting survey.

Although both these aims were achieved to a certain extent by trend analysis, the technique was limited by the need for computing facilities. Much simpler criteria were needed.

Three different approaches to the problem were made and these are discussed below.

2. The use of data distributions.

(a) Introduction.

Liebscher and Smith (1968) in discussing essential and non-essential elements in human tissues suggested that essential elements show normal distributions and that non-essential elements show log-normal distributions. One of the criteria these authors used to determine which type of distribution best fitted the data, was a comparison of the arithmetic and geometric means with the median. Log-normal distributions have the same value for the geometric mean and the median, while for normal distributions, the arithmetic mean and the median are the same.

To test the postulate of Liebscher and Smith for the case of plants, the two means and the medians were determined for all the leaf ash data collected in the course of this thesis. In addition, analytical results published by other workers were used to further test the theory.

(b) Results.

The ratios of both the arithmetic and the geometric mean to the median are given in Table IV - 18 for all the species and metals considered. It can be seen from the table that for most metals, whether they are considered essential or not, the geometric means were closer than the arithmetic means to the medians. This implied that nearly all the data distributions were more log-normal than normal.

(c) Discussion.

It was apparent from these results that the use of normal and log-normal distributions to distinguish essential from non-essential metals, although of use for human tissues, was not sufficiently reliable to be of any value for the plant samples considered in the present study. This was probably due to the fact that most of the data presented in Table IV - 18 were influenced by anomalous concentrations of the metals in the soils. This caused the distributions of the essential metals to be skewed away from their usual distributions which may, in fact, be close to normal.

3. The coefficients of variation.

(a) Introduction.

It was observed from the frequency histograms of the data sets presented in Table IV - 18, that the essential metals tended to cover much narrower concentration ranges in the plants than did the non-essential metals. This indicated the possibility of using the coefficients of variation as indicators of essential and non-essential behaviour i.e. a low coefficient of variation for the concentration of a particular metal in a certain species of plant would indicate the essential nature of the metal and the probable poor results that would be obtained from a biogeochemical survey for this metal. However, it was also possible that a narrow concentration range in the plants could be the result of a narrow concentration range in the soil. There were, therefore, a number of possible situations:

TABLE IV - 18

Ratios of the geometric and arithmetic means to the medians for various metals in different plant species.

Species	No. of Samples	Data + Source	Ni		Cu		Zn		Pb		Mo		U	
			A	B	A	B	A	B	A	B	A	B	A	B
N.fusca	89	a	1.07	1.39	1.06	1.26	1.04	1.08						
	26	c	-	-	-	-	-	-	-	-	-	-	0.60	1.12
N.menziesii	70	a	0.98	1.21	1.13	1.51	1.04	1.12	-	-	-	-	-	-
N.truncata	57	a	1.00	1.14	1.06	1.21	0.98	1.01	-	-	-	-	-	-
W.racemosa	94	b	1.02	1.19	1.02	1.06	0.96	1.06	-	-	-	-	-	-
	20	c	-	-	-	-	-	-	-	-	-	-	0.60	1.65
Q.acutifolia	88	b	1.02	1.09	1.04	1.11	0.96	0.98	-	-	-	-	-	-
	22	c	-	-	-	-	-	-	-	-	-	-	0.47	2.60
Olearia rani	71	d	-	-	1.07	1.35	-	-	-	-	0.93	2.90	-	-
Schefflera digitata	36	e	-	-	1.06	1.38	1.14	1.34	1.03	1.45	-	-	-	-
Beilschemedia tawa	35	e	-	-	1.00	1.32	1.04	1.13	1.23	2.29	-	-	-	-
Triodia pungens	30	f	-	-	0.98	1.07	-	-	-	-	-	-	-	-

\+ a this thesis (includes all samples collected)
b this thesis March, 1969, samples
c Whitehead and Brooks (1969)
d Lyon *et al*.(1968)
e Nicolas and Brooks (1969)
f Nicolls *et al*.(1965)

A = geometric mean/median
B = arithmetic mean/median

(1) A narrow concentration range in the plants relative to the concentration range in the soil, would indicate an essential metal and imply poor biogeochemical results.

(2) A wide concentration range in the plants relative to the concentration range in the soil would indicate a non-essential metal and imply good biogeochemical results.

(3) Similar concentration ranges in both the plants and the soil could indicate either an essential or non-essential metal but could not indicate the outcome of a biogeochemical survey.

It was therefore necessary to select a value for the ratio of the coefficients of variation of the plant data to the soil data, which would result in an accurate prediction of the successfulness of a biogeochemical prospecting survey. The arithmetric coefficients of variation of the plant and soil data as well as the ratio of one to the other are given in Table IV - 19.

(b) Results.

If a value of 0.70 for the ratio of the coefficients of variation was selected, above which non-essential metals were involved and below which essential metals were involved, then the prediction of non-essentiality was correct in eight out of ten cases. Due to the very small concentrations of molybdenum actually necessary for plant nutrition it was considered non-essential in the concentration ranges being considered. The prediction of essentiality was however correct in only eight out of sixteen cases.

TABLE IV - 19

Coefficients of variation for some metal concentrations in the leaf ash of various plant species and their associated soils.

Metal	Species	Data+ Source	No. of Samples	Coefficients of Variation			Correlation Coefficients
				Plant	Soil	Plant/ Soil	Plant: Soil
Nickel	W.racemosa	b	94	62	43	1.44	0.43^{++}
	Q.acutifolia	b	88	41	44	0.93	0.41^{++}
	N.fusca	a	89	95	52	1.83	0.612^{++}
	N.truncata	a	57	54	38	1.42	0.528^{++}
	N.menziesii	a	70	65	60	1.08	0.474^{++}
Copper	W.racemosa	b	94	30	101	0.30	0.05
	Q.acutifolia	b	88	48	126	0.38	0.36^{++}
	B.tawa	e	35	135	253	0.54	-0.03
	S.digitata	e	36	62	192	0.32	-0.03
	O.rani	d	71	61	54	1.13	0.08
	T.pungens	f	30	43	114	0.38	0.53^{++}
	N.fusca	a	89	127	110	1.15	0.294^{+}
	N.truncata	a	57	95	133	0.71	0.344^{+}
	N.menziesii	a	70	137	78	1.76	0.163
Zinc	W.racemosa	b	94	30	30	1.00	0.02
	Q.acutifolia	b	88	21	30	0.70	0.03
	B.tawa	e	35	39	169	0.23	0.06
	S.digitata	e	36	71	135	0.53	0.41^{+}
	N.fusca	a	89	31	25	1.24	0.319^{+}
	N.truncata	a	57	26	20	1.30	-0.073
	N.menziesii	a	70	42	31	1.35	0.155
Lead	B.tawa	e	35	157	248	0.63	0.51
	S.digitata	e	36	78	207	0.38	0.28
Molybdenum	O.rani	d	71	252	65	3.87	0.74^{++}
Uranium	W.racemosa	c	20	130	129	1.00	0.72^{++}
	N.fusca	c	26	100	121	0.82	0.62^{++}

Levels of significance ++ < 0.001
+ < 0.01

+ Data source as for Table IV - 18

(c) Discussion.

The poor results obtained above could be attributed to two factors:

(1) The range of zinc concentrations in the soil were similar to the range in the plants for five examples, and, as mentioned earlier, the method was not expected to predict essentiality in this case.

(2) In the case of copper, a few anomalously high concentrations in the leaf ash were recorded for the Nothofagus species with the result that the standard deviations were abnormally large, and hence high ratios of the coefficients of variation were obtained for copper leading to the incorrect classification.

Overall, the prediction of non-essential and essential nature was correct in sixteen out of twentysix cases.

The second aim of the study was to try and predict the outcome of a biogeochemical prospecting survey involving a particular plant and metal system. If the value of 0.70 for the ratio of the coefficients of variation was used, then values greater than this were found to predict significant correlations in seventeen cases. Out of these seventeen only eleven were correct. Also this criterion failed to predict three cases of significant correlations.

In conclusion, it was apparent that the accuracy of the technique was not sufficiently high to make it a promising tool to predict the outcome of a biogeochemical prospecting survey.

4. The relative accumulation of a metal as a function of the total concentration of the metal in the soil.

(a) Introduction.

Since plants tend to contain constant concentrations of metals essential to their nutrition, then where the soil concentrations of these metals vary, the relative accumulations must vary inversely to the concentration in the soil i.e. this could be readily detected by a negative correlation coefficient. The appropriate correlation coefficients were computed for all the available data and are shown in Table IV - 20.

(b) Results.

In every case a negative correlation was obtained. It was apparent from these results that for both copper and nickel, the least negative correlations were shown by the *Nothofagus* species which was also found to be the best species for prospecting purposes (see plantxsoil correlation coefficients in Table IV - 20). This observation was expected however, since a highly significant negative relationship for the relative accumulation correlated with the total soil concentration, implied a constant plant concentration and hence a poor plant/metal system for biogeochemical prospecting.

TABLE IV - 20

Correlation coefficients between the relative accumulations and the total metal concentrations in the soil.

Species	No. of Samples	Correlation coefficients +					
		Nickel		Copper		Zinc	
N. truncata	57	-0.479	(0.528)	-0.884	(0.344)	-0.633	(-0.078)
N. fusca	89	-0.339	(0.612)	-0.841	(0.294)	-0.583	(0.319)
N. menziesii	70	-0.424	(0.474)	-0.801	(0.163)	-0.700	(0.195)
Q. acutifolia	88	-0.731	(0.411)	-0.924	(0.355)	-0.778	(0.001)
W. racemosa	94	-0.519	(0.427)	-0.944	(0.054)	-0.655	(0.016)

\+ Correlation coefficients in brackets are those for the corresponding plant×soil relationships.

(c) Discussion.

In SECTION IV - E it was shown that the accumulation of nickel decreased as the nickel concentration in the soil increased, which was in agreement with the correlations obtained in this section. This was interpreted as being the result of an exclusion of nickel from the plant in regions of high soluble nickel concentrations in the soil, possibly due to a metabolic exclusion process or to competition with other metal ions.

For the case of copper, the accumulation also decreased as the concentration of copper in the soil increased. But this was considered rather as an increase in relative accumulation as the soil concentration decreased and was interpreted as being due to the metabolic accumulation of copper from soils low in copper, to satisfy the requirements of the plants for this metal. Zinc is also essential for plant nutrition and the reasons for the results obtained were similar to those for copper.

Since all the data sets were transformed to base ten logarithms before correlation, the highly significant negative relationships observed implied a tendency towards rectangular hyperbolic behaviour for the untransformed data. This is shown in Figure IV - 8 where the graphs of the relative accumulations versus total soil concentrations for copper and zinc in Q.acutifolia leaf ash are presented. The graphs of these metals are in contrast to that for nickel in N.fusca leaf ash also shown in Figure IV - 8.

For biogeochemical prospecting results to be successful, the relative accumulation of a metal by a plant must be indepen-

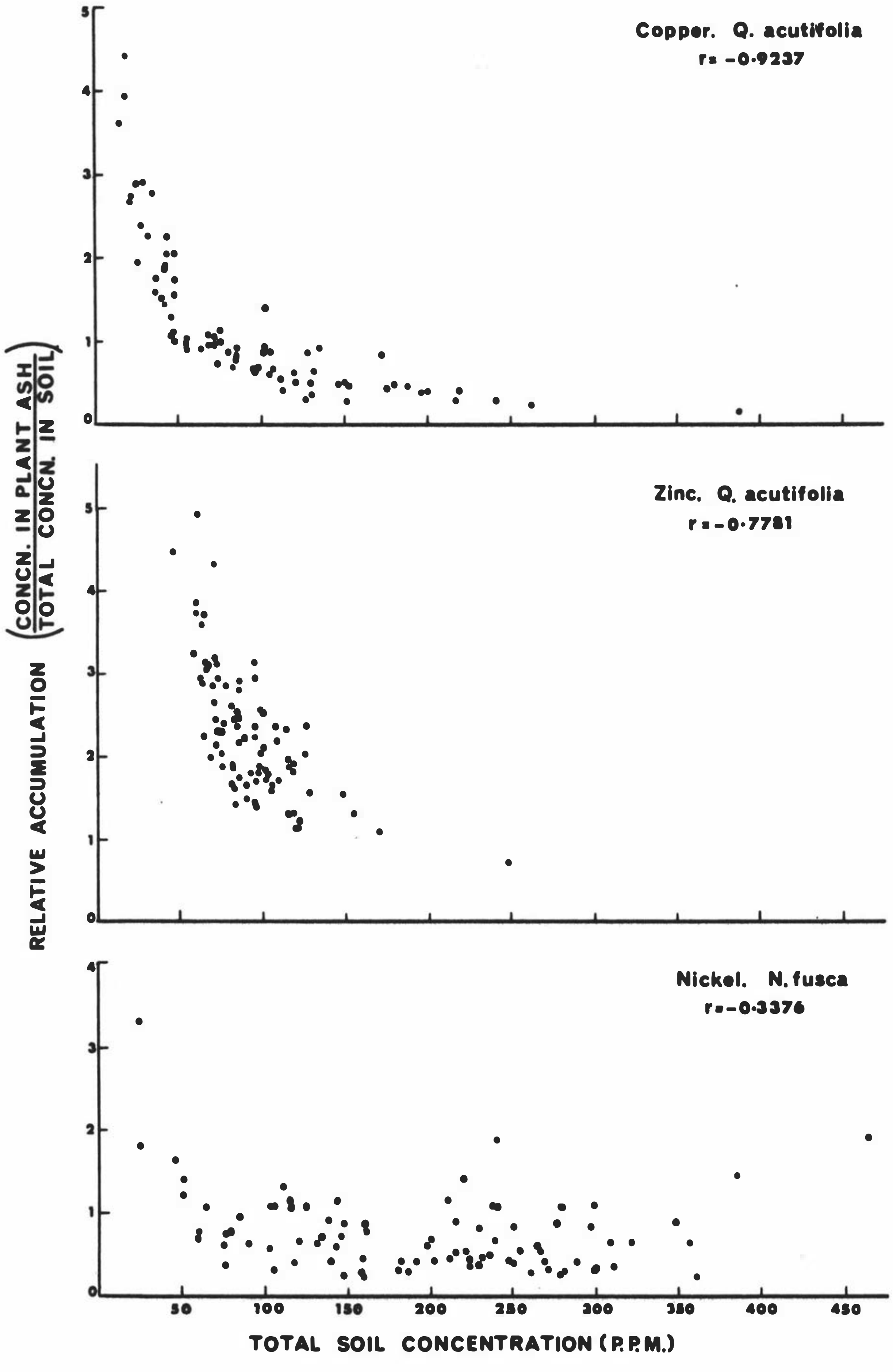


Figure IV-8 Relative accumulation versus total soil concentration.

dent of (or proportional to) the metal concentration in the soil.

From Figure IV - 8 however, it can be seen that for copper in the leaves of Q.acutifolia this criterion was not satisfied until the copper concentration in the soil exceeded 100p.p.m. Only above this level did the relative accumulation become approximately independent of the soil concentration.

These observations can be readily interpreted depending on the concentrations of copper in the soil. Below approximately 100p.p.m. copper in the soil, Q.acutifolia was metabolically accumulating copper to satisfy its requirements. For this species, the level required in the dry leaf was approximately 2 to 4p.p.m. or 40 to 80p.p.m. in the leaf ash. Above approximately 100p.p.m. copper in the soil, Q.acutifolia no longer accumulated copper to the same extent and probably started to exclude it. But it was apparent that the exclusion of copper by the plants was not entirely efficient since where soils contained anomalous concentrations of copper, some leaf concentrations, particularly for the Nothofagus species, were considerably higher than normal.

The same reasoning can be applied to the case of zinc (Figure IV - 8), although no soil concentrations were observed for which the relative accumulation tended to become constant. This was because there was no zinc mineralisation in the study area.

For the case of nickel however, no marked deviation away from constant relative accumulation was observed except for

Q.acutifolia which showed a negative correlation of -0.731 (Table IV - 20). The reason for this is not clear but the results indicated that this species, and to a lesser extent the other species, tend to maintain a minimum amount of nickel in their tissues. The graph presented in Figure IV - 3 for nickel in *N.fusca* contains three points which deviate from the general population. One point at a relative accumulation of 3.3 and a soil concentration of 20p.p.m. was due to a plant containing a nickel concentration indicative of a basic substrate while the corresponding soil showed sedimentary characteristics. There is little doubt that this point caused the negative correlation observed.

The negative correlation coefficient for the other species were also partly due to points such as this one. Two other points of high relative accumulation and high soil concentrations (>375p.p.m.) are worthy of mention because of their apparent anomalous behaviour. Although it was not investigated further, it was thought that the samples corresponding to these points were contaminated to a small extent by soil. In general, the relative accumulation values for nickel showed a less regular relationship to the nickel content of the soil than was observed between the relative accumulations of copper and zinc and the concentrations of these metals in the soils. This was probably due to the regulation by the plants of the accumulations of copper and zinc in contrast to the accumulation of nickel, which was not regulated by the plants.

(d) Conclusions.

It was concluded from these studies that for metal/plant systems, a graph of the relative accumulation versus the total concentration in the soil is of particular value, for it shows at a glance the lowest metal concentration in the soil below which biogeochemical prospecting results would be unreliable. If this minimum soil concentration is not significantly below the soil concentration expected to indicate mineralisation, then there is little point in carrying out a biogeochemical survey. In addition, from the relative accumulation values lying on the curved part of the graph some indication can be obtained of the optimum amount of the metal required by the plant. For example, in the case of copper in *Q.acutifolia* leaf ash, this amount is approximately 40 to 80p.p.m. (c.f. the result of 105p.p.m. in the leaf ash of the *Nothofagus* species, obtained by trend analysis in SECTION IV - B).

SECTION V

TRACE METAL COMPLEXES IN LEAVES

A. INTRODUCTION.

In the introduction to this thesis it was pointed out that there are two approaches to the study of biogeochemical processes; the direct experimental approach which is the most common and the statistical approach which has been illustrated in the previous sections of this thesis. Although the judicious application of statistics to observed data can, in some cases, serve to detect relationships among trace metals in plants and soils, further elucidation of the relationships relies heavily on the experimental approach.

There are, however, a number of problems inherent in the experimental approach to both plant and animal systems. In the past, most metals administered to organisms contained radioactive isotopes as tracers (Broda, 1960). Although the use of radioisotopes is a very sensitive method, free from contamination problems and usually very convenient, attempts to study metals in tissues at very low concentrations are often limited by the specific activity of the cation solution administered to the organism. The need for a useful specific activity in the tissues may require the administration of potentially toxic concentrations of the metal.

There are other disadvantages, particularly with the use of radioisotopes which emit low energy radiation. For example, the only useful long-lived isotope of nickel is nickel - 63 which emits a B particle of energy 0.067Mev. The organism may accumulate such low concentrations of this metal that measurement of the radiation in the tissues is very difficult.

Another well-known problem arises when only short-lived isotopes are available such as the copper - 64 isotope with a half-life of 12.9 hours. The short half-life is this isotope is probably one of the main reasons for the relative lack of literature on the metabolism of this metal in plants as compared with iron and, to a lesser extent, zinc.

In view of the above comments it is apparent that while radioactive tracers will continue to play the dominant role in trace metal studies, there is an urgent need for methods not based on radioactivity and involving total analysis of metal concentrations in the submicrogram range.

In addition to the application of atomic absorption spectrophotometry described below, other sensitive methods of analysis for trace metals have recently been developed. These methods generally depend on the principles of atomic absorption or atomic fluorescence spectrophotometry. Such systems which have been reported include the carbon-rod atomizer (West and Williams, 1969; Amos *et al.*, 1971), the graphite-tube atomizer (Massmann, 1968; Manning and Fernandez, 1970) and the platinum-loop atomizer (Bratzel *et al.*, 1970). The absolute detection limits which are possible with these techniques are very low. For example, the detection limits obtainable with a Varian-Techtron carbon-rod atomizer Model 61 are 10^{-11} gm of nickel and 5×10^{-12} gm of chromium. When these values are compared to the detection limits attained in this thesis for these two metals, 2×10^{-8} gm, for nickel and 3×10^{-8} gm for chromium (Table II - 1) using conventional atomic absorption spectrophotometry, it is apparent that the new methods offer considerable promise for application to biological systems.

Although the application of such analytical techniques does not present a great problem, the difficulties of contamination do. In the use of radioactive tracers, contamination from other sources of radioactivity is unlikely. On the other hand, in the total analysis of metals at the submicrogram level, contamination is very difficult to avoid. However, stringent precautions during the experimental manipulations can reduce this problem to an acceptable level.

The present section describes a brief study of the location and chemical forms of nickel in the foliage of some of the trees growing in the sampling area. The lack of knowledge concerning the chemical forms of the "available" nickel in the soil of the area presented a major obstacle to the setting up of experiments involving plants grown in nutrient solutions. It was possible that the conditions existing in the soil of the Complex may have been difficult to duplicate in the laboratory. To overcome this difficulty, leaf tissue collected from trees growing on the Complex was used in the present study. This tissue contained nickel accumulated under natural conditions in sufficient concentrations to allow the use of atomic absorption spectrophotometry for its detection and measurement.

B. EXPERIMENTAL.

1. Sample collection.

The leaf samples used in this study were collected from two specimens of each of the species N.fusca, W.racemosa and Q.acutifolia. In addition, leaves were taken from one specimen of Pseudowintera colorata which was growing within a few feet of the sulphide outcrop at site (2,8).

The locations at which the samples were collected are given in Table V - 1. Values for the exchangeable concentrations of nickel and copper in the soils at each location are also given in Table V - 1. These values were obtained by extracting 4.0gm of soil with 10.0ml of various extractants.

Approximately 1 to 2lbs of leaves were removed from each tree and transported in plastic bags back to the laboratory where the leaves were washed gently in tap water, followed by distilled water. The quantities necessary for the differential centrifugation experiments were stored at 4°C for approximately 36 hours, while the remainder were freeze-dried, ground to less than 1mm in a hammer mill, and stored in air-tight brown glass jars at -10°C.

2. Chemicals and Glassware.

Since quantitative measurements were made of metals in the microgram concentration range, all solvents and chemicals had to be exceptionally pure.

TABLE V - 1

Details of the leaf samples used in the study of the metal complexes in leaves.

			Metal concentrations in the soils (p.p.m.)					
		Sampling	Nickel			Copper		
Species	Specimen	Location	A+	B	C	A	B	C
N.fusca	b	(2,3)	404	0.16	2.6	191	0.17	4.0
W.racemosa	b							
Q.acutifolia	b							
W.racemosa	a	(5,7)	476	0.29	2.2	884	0.61	12.0
N.fusca	a	(2,8)	550	6.4	20.1	1800	30.0	465.0
Q.acutifolia	a							
P.colorata	a							

\+ A = Total (-120 mesh)

B = 1M ammonium acetate

C = 0.05M disodium E.D.T.A.

N.B. Soils were typical of those in the vicinity of each site, they were not collected immediately below the trees from which leaf samples were taken.

Solvents were distilled concentrated approximately 20 times and analysed for nickel, copper, zinc and iron. Distillation was repeated until the concentrations of these metals were below the detection limit of the atomic absorption spectrophometer (Table II - 1). The chemicals were all of analytical grade and contributed negligible amounts of nickel, copper, zinc and iron to the solutions in which they were used.

Decontamination of glassware was most effectively achieved by washing with a commercial detergent to remove organic material followed by boiling in dilute, metal-free hydrochloric acid. Reabsorption of nickel on to the active glass surface during the extractions of leaf tissue was found to be negligible. (c.f. percentage recoveries in Table V - 2.)

C. DIFFERENTIAL CENTRIFUGATION OF FRESH LEAVES.

1. Method.

For a preliminary study of the subcellular distribution of nickel in the leaves of the plant species used in this study it was not considered necessary to undertake extensive experimentation to determine the optimum conditions for separation. Instead, a standard procedure was adopted (Stern, 1968). Although this method was intended primarily for soft leaves (grasses etc.), it proved to be suitable for the present study with only minor modifications to the preparation stage.

The fractions obtained and their major components are given in Figure V - 1.

The residue obtained at 1000g for 10 minutes contained both cell wall fragments and chloroplasts but no further separation of these components was attempted.

2. Procedure.

The leaf samples used were collected in the field, transported to the laboratory, washed and placed in cool storage within one day. Three samples were used; one each of *N.fusca*, *Q.acutifolia* and *P.colorata*. These samples were all classified as specimen "a".

For the large leaves of *Q.acutifolia* and *P.colorata*, it was necessary to remove the midribs from the leaf blades before blending.

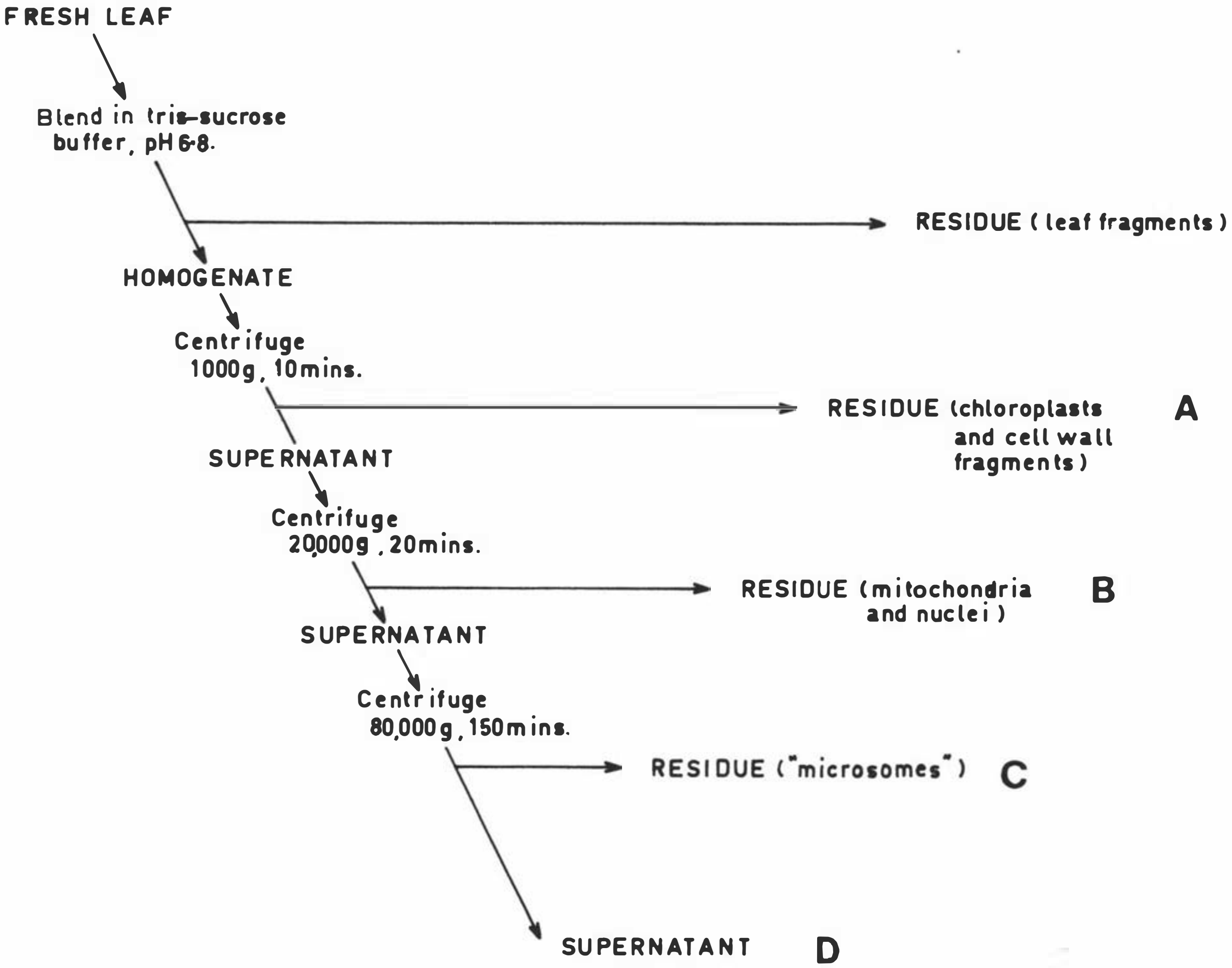


Figure V-1. Differential centrifugation scheme (after Stern, 1968)

Twenty gm of leaf tissue was blended in a Waring Blendor at full speed for 18 x 10 seconds in 200ml of tris-sucrose buffer, pH6.8 (0.05M trishydroxymethylaminomethane - HCl, 0.5M sucrose, pH adjusted with acetic acid). All manipulations were carried out at 0 - 4°C.

The resulting pulp was strained through a 125 micron nylon mesh and the filtrate separated into five or six equal portions. All portions were centrifuged to give fractions A, B and C + D. The C + D fractions of two portions were further separated to give fractions C and D. The results presented are the means of the A, B, C and C + D fractions.

3. Analytical method.

Because of the large quantities of leaf tissue used, it was found that by drying each fraction at 80°C, ashing at 430°C and dissolving in 1M hydrochloric acid, reproducible results were obtained. The acid solution was analysed directly by atomic absorption spectrophotometry.

4. Results.

All fractions for each sample were analysed for nickel, copper, zinc and iron and the results are presented in Table V - 2. Two samples of *Q.acutifolia* were subjected to the separation scheme to give a measure of the precision of the separation.

These results are also shown in Table V - 2.

By consideration of the values obtained for the two replicates of *Q.acutifolia*, the approximate errors in precision were, nickel 1%, copper 9%, zinc 3%, and iron 4%.

Approximately 90% of the nickel in all samples remained in the supernatant fraction after centrifuging at 80,000g for 150 minutes. For both copper and zinc, 50% to 70% remained in the supernatant fraction, while only 10% to 15% of the iron was present in this fraction. For most samples, less than 20% of each metal was present in fractions B and C.

Within the error limits, all species showed the same percentage fractionation of nickel and copper. For zinc, *P.colorata* and *N.fusca* contained a higher percentage in their supernatant fraction than did *Q.acutifolia*. In the case of iron, the only apparent difference was that *N.fusca* and *P.colorata* contained a higher ratio in fraction B relative to fraction A than was found for *Q.acutifolia*.

5. Discussion.

(a) Nickel.

Although the separation of the cell components was probably not complete, the results obtained suggested that in all the species studied, approximately 90% of the total nickel was not contained in the cell organelles. This nickel was most probably present in the leaf either in the cytoplasm or in the vacuole.

TABLE V - 2

Percentage distributions of some metals in the fractions obtained by differential centrifugation of the fresh leaves.

Metal	Species	Total concn $\mu g/gm$	Replicate No.	Fractions +			
				A	B	C	C + D
Nickel	N.fusca	8.05	1	8.25 (6)	2.35 (6)	1.42 (2)	89.0 (4)
	P.colorata	14.7	1	8.00 (5)	2.48 (5)	1.65 (2)	89.5 (3)
	Q.acutifolia	6.20	1	9.65 (6)	0.36 (6)	2.53 (2)	90.0 (4)
			2	10.3 (5)	0.21 (5)	-	89.5 (5)
Copper	N.fusca	5.10	1	33.4 (6)	7.53 (6)	5.30 (2)	59.0 (4)
	P.colorata	5.10	1	34.0 (5)	9.70 (5)	2.70 (2)	56.2 (3)
	Q.acutifolia	2.03	1	39.6 (6)	0.60 (6)	8.30 (2)	59.7 (4)
			2	45.2 (5)	3.38 (5)	-	51.3 (5)
Zinc	N.fusca	8.56	1	23.2 (6)	8.40 (6)	3.90 (2)	63.5 (4)
	P.colorata	17.9	1	21.8 (5)	7.44 (5)	3.60 (2)	70.8 (3)
	Q.acutifolia	7.20	1	39.4 (6)	8.35 (6)	6.00 (2)	52.1 (4)
			2	38.6 (5)	6.29 (5)	-	55.0 (5)
Iron	N.fusca	41.5	1	70.7 (6)	18.3 (6)	5.51 (2)	11.0 (4)
	P.colorata	28.5	1	77.0 (5)	12.6 (5)	1.75 (2)	10.5 (3)
	Q.acutifolia	18.3	1	80.0 (6)	4.77 (6)	5.23 (2)	15.3 (4)
			2	83.4 (5)	4.10 (5)	-	12.6 (5)

\+ A "cell wall fragments/chloroplasts"

B "mitochondria and nuclei"

C "microsomes"

D supernatant

The remaining 10% of the total nickel was found in the fraction containing the cell wall fragments and the chloroplasts but since no further separation of these components was made, the precise location was not known.

These results were in reasonable agreement with the solvent extraction results presented in Part D of this section, if the 10% nickel remaining in the cell wall/ chloroplast fraction was considered to correspond to the similar amount of nickel remaining in the residue after solvent extraction.

(b) Copper, zinc and iron.

The percentages of these metals in the chloroplast / cell wall fraction varied from about 25% for zinc to 85% for iron in contrast to the 10% of the total nickel observed in this fraction.

The percentage copper in thisfraction was essentially the same for all species with between 30% to 40% of the total copper present. This value is lower than that observed by Neish (1939) who found 74.6% of the total copper in the leaves of Trifolium pratense to be in the chloroplasts. The low values observed in the present study may be due to the fact that the leaf samples all came from trees growing in relatively high soil concentrations of copper (Table V - 1). This caused higher concentrations in these trees e.g. approximately 10 - 15p.p.m. on a dry weight basis, compared to the mean values in the trees over the grid area of approximately 3 - 5p.p.m.(Table III-3).

The copper accumulated by the plants in excess of that required for the chloroplasts would be present in the supernatant fraction.

A difference in the distribution of zinc was observed between N.fusca and P.colorata on the one hand and Q.acutifolia on the other. The former two species showed slightly lower concentrations in the chloroplast/ cell wall fraction and higher concentrations in the supernatant fraction than was observed for Q.acutifolia.

Iron was shown to exist almost entirely in the cell wall/ chloroplast fraction of all the species studied. This finding was in agreement with previous studies by Liebich (1941) who found up to 82% of the total iron in Spinacia oleracea to be in the chloroplasts, and Jacobson (1945) who showed that 61% to 81% of the total iron in green tobacco leaves was present in the chloroplasts.

In the plants studied in this thesis, copper, zinc and iron were present in only small amounts in the "mitochondria" and "microsome" fractions and in view of the possibility of contamination from the other fractions, the significance of these small amounts could not be assessed.

D. SOLVENT EXTRACTION OF NICKEL FROM FREEZE-DRIED LEAF TISSUE.

1. Extraction procedure.

The scheme followed in this work was adapted from the procedure proposed by Bowen *et al.* (1962). For a preliminary comparison of the chemical nature of nickel in each sample of leaf tissue, the five-fraction separation scheme shown in Figure V - 2 was used.

Trial experiments showed that 1gm of freeze-dried tissue could be readily dealt with by this extraction procedure and the analytical technique described below. Sufficient quantities of all fractions were obtained for quantitative analysis and further separation by electrophoresis or chromatography.

2. Analytical method.

Each fraction was taken to dryness under reduced pressure (35°C) and redissolved in 5.0ml of the appropriate solvent. For the analysis of each extract, 3 x 1ml aliquots were added to three test tubes, each containing one circle of Whatman (No.541, 7cm) filter paper. The contents of the test tubes were dried at 80°C and ashed at 430°C in a muffle furnace.

Drying and ashing in the absence of filter paper, resulted in a poor recovery of nickel, probably due to the residue adhering to the glass walls of the tube. With filter paper present, at 80°C the solvent evaporated more rapidly from the filter paper than from the solution surface, with the result that almost all the solution

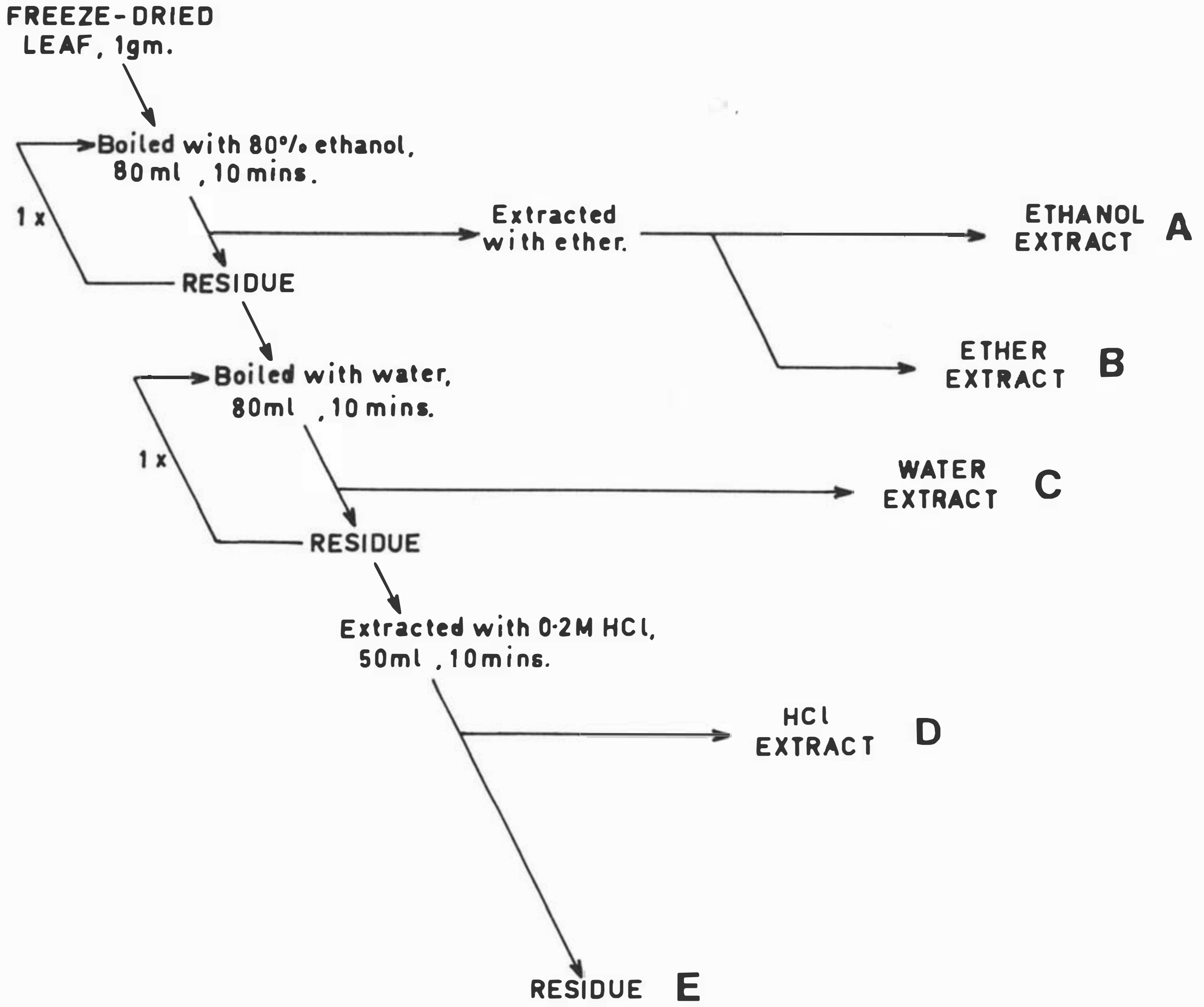


Figure V-2. Solvent extraction scheme for freeze-dried leaf tissue.
(modified from Bowen *et al.*, 1962)

was absorbed up into the filter paper before drying was complete. On ashing, a small pellet of ash remained which contained the metals from the extract and those from the paper. This ash was dissolved in 1ml of 1 M hydrochloric acid and the solution analysed.

The means of the three determinations for each extract were taken which agreed within ±5% and usually within ±2%. No blank correction for the filter paper was necessary.

The total quantity of nickel recovered from the Bowen extraction was expressed as a percentage of the original amount present in the quantity of tissue used (1gm).

3. Results.

Two portions of each sample were subjected to the extraction procedure and the results for both are presented in Table V - 3 to illustrate the precision of the method.

These results showed that in all samples, most of the nickel present was soluble in the aqueous ethanol and water fractions. Approximately 85.0% was extracted into these fractions from the samples of N.fusca, 75% from the sample of P.colorata and 60% to 70% from the samples of Q.acutifolia and W.racemosa. Of the nickel remaining in the residue after extraction by aqueous ethanol and water, the ratio of acid soluble to insoluble ranged from approximately 1.0 for N.fusca and P.colorata samples, to 2.0 and 4.0 for the samples of W.racemosa and Q.acutifolia respectively.

TABLE V - 3

Percentage distributions of nickel in the freeze-dried leaves among the various solvent extracts.

Species	Specimen	Total ** nickel concn.	Fraction *						% Recovery
			A	B	C	A+B+C	D	E	
W.racemosa	a	11.9	48.1	5.1	14.3	67.5	21.1	11.4	97.0
			43.2	0.0	25.2	68.4	18.0	13.5	93.3
	b	10.3	36.4	0.0	32.3	68.7	27.3	4.1	95.2
			35.7	0.0	25.5	61.2	24.5	12.3	96.0
N.fusca	b	6.9	68.0	1.9	15.1	85.0	9.5	5.7	107.[illegible]
			65.5	3.4	17.2	86.1	10.3	3.0	117.0
	a	14.5	57.1	1.4	25.7	84.2	7.2	8.6	97.2
			54.1	4.2	26.4	84.7	7.0	8.3	99.5
Q.acutifolia	b	5.0	34.4	0.0	27.2	61.6	31.7	6.8	88.5
			31.2	0.0	26.2	57.4	34.4	8.2	120.0
	a	10.5	33.2	0.0	33.2	66.4	26.2	7.4	91.7
			34.3	0.0	28.6	62.9	28.6	8.6	100.0
P.colorata	a	29.0	42.3	1.3	32.9	76.5	12.8	10.7	103.0
			43.0	0.0	32.2	75.2	14.1	10.7	103.0

* Values expressed as percentage of the total recovered.

A 80% aqueous ethanol

B ether

C water

D 0.2 M hydrochloric acid

E residue

** micrograms/gram of freeze-dried tissue

N.B. Two replicates of each sample and two samples of each species except P.colorata are shown.

A very small amount of nickel was extracted into the ether phase for some samples but it is likely to have arisen because of incomplete separation from the aqueous ethanol fraction.

4. Discussion.

The main constituents of the five fractions were as follows:

A - 80% aqueous ethanol - amino acids, lipids, moderately polar metal complexes.

B - ether - pigments, high molecular weight organic acids.

C - water - ionic and strongly polar compounds.

D - 0.2 M hydrochloric acid - pectates, proteins and exchangeable cations.

E - residue - structural components, e.g. cellulose, hemicellulose, lignin and some proteins and nucleic acids.

The results observed suggested that the major part of the nickel present in the leaf tissues (60 - 80%) occurred as a polar compound(s), possibly of low molecular weight. Differences observed between the species were not large and may be due to differences in the ease of extraction resulting from varying extents of grinding of the leaf samples. An additional smaller fraction was found to be extractable in 0.2 M hydrochloric acid, which could possibly indicate that the nickel was bound to the proteins or pectates extracted with this solvent. Alternatively, the nickel present in the acid extract was present as the free divalent cation which had dissociated from compounds in the residue under the acid conditions. For example, Magnus (1958) showed that in the pH range 2.0 to 5.0 no nickel coordinated to the proteins insulin, calf thymus histone and kerateine (a partially degraded product from lambs wool).

Unfortunately, there are no other reports in the literature on nickel with which to compare the present observations. Chromium, another apparently non-essential metal to plant nutrition, has been studied and Lyon *et al.* (1969a) showed that 15.4% of the total chromium in the shoots of *Leptospermum scoparium* was soluble in 80% aqueous ethanol and water, while a further 78.0% was extractable with 0.5 M perchloric acid and 2 M sodium hydroxide. This was in contrast to the results for nickel obtained in the present study. Copper and zinc have received some attention, primarily because of their essential nature to both plant and human nutrition. Bowen *et al.* (1962) showed that in freeze-dried tomato tissues, 9.1% of the total copper and 5.8% of the total zinc were soluble in 80% aqueous ethanol, and 73.4% and 84.4% of these metals respectively were extractable into 0.2 M hydrochloric acid. This author also noted that 6.2% of the copper occurred in the acids extracted into the ether phase. Peterson (1969) found that in *Agrostis tenuis* between 52.9% and 66.7% of the total zinc in the shoots, depending on the level of zinc in the nutrient solution, could be extracted with boiling 80% aqueous ethanol and water. Bremner and Knight (1970) observed that 65% of the total zinc in fresh ryegrass was extractable by cold 80% aqueous ethanol and water. Diez-Altares and Bornemisza (1967) studied the form of zinc in corn seedlings and found that approximately 70% of the total zinc was extractable with 70% aqueous ethanol, molar sodium chloride and 0.05 M sodium hydroxide.

It is apparent that considerable differences in the solubilities of copper and zinc occur between species. This makes useful comparisons difficult until the chemical forms of the metals are known. However, it seems that in the plants studied by the workers mentioned above, most of the copper and zinc in the leaf tissues was in a soluble or readily-exchangeable form. This is in agreement with the present findings for nickel.

E. HIGH-VOLTAGE ELECTROPHORESIS OF THE 80% AQUEOUS ETHANOL EXTRACTS FROM FREEZE-DRIED LEAVES.

1. Procedure.

Paper electrophoresis was carried out on a high-voltage apparatus (Miles Hivolt) using Whatman 3MM, 43cm x 53.5cm, paper.

Since the stabilities of any organo-nickel complexes present in the extracts were unknown, it was considered necessary to maintain the buffer pH at a level similar to that expected to exist in the leaf tissue. A pyridine/ acetic acid buffer pH 5.3 was used.(Efron, 1960)

Papers were dipped in buffer, blotted evenly to remove surplus liquid, and placed on the bed of the instrument. Concentrated extract (50µl to 100µl) was applied with a glass microlitre pipette as a 35cm streak across the centre of the paper and a potential of 6kv. at a current of 380 ± 10ma was maintained for 10 minutes. Heat was dissipated by circulating water in the pressure plates above and below the paper.

The papers, after removal from the apparatus, were dried rapidly in a stream of warm air.

2. Analytical method.

The electrophoresis papers were cut into strips 1cm wide parallel to the origin streak. Each strip was placed in a separate test tube and ashed at 430°C. For maximum sensitivity 0.3ml of hydrochloric acid was added to each tube.

The nickel in each tube was determined quantitatively by atomic absorption spectrophometry. This gave the concentration of nickel on the paper at points 1cm apart along the direction of movement.

3. Results.

The analyses of the electrophoretograms are shown in Figure V - 3. Each scan is the average of two electrophoresis papers.

It was found that due to the high amounts of organic material, compared to the amount of nickel in the extract, the resolution of nickel on the papers was very poor in most cases. The results, however, indicated the presence of a positive nickel compound in the aqueous ethanol extracts of all leaf samples. The aqueous extracts showed similar electrophoretic patterns. In addition to the mobile nickel compound some nickel remained on the origin in every case. It was noticed, however, that the ratio of mobile to immobile nickel was variable and increased as the total quantity of extract applied to the paper was decreased.

The electrophoretic pattern of the divalent nickel cation is also shown. This ion migrated further than the nickel compounds in the leaf extracts.

Figure V - 3 Patterns of nickel after electrophoresis at pH 5.3 of the 80% aqueous ethanol leaf extracts.

A	*N.fusca*	(a)
B	*N.fusca*	(b)
C	*Q.acutifolia*	(a)
D	*Q.acutifolia*	(b)
E	Standard Ni^{2+}	
F	*W.racemosa*	(a)
G	*W.racemosa*	(b)
H	*P.colorata*	(a)

N.B. All electrophoretograms were run on separate papers, hence the small differences in the migration distances of the nickel compound.

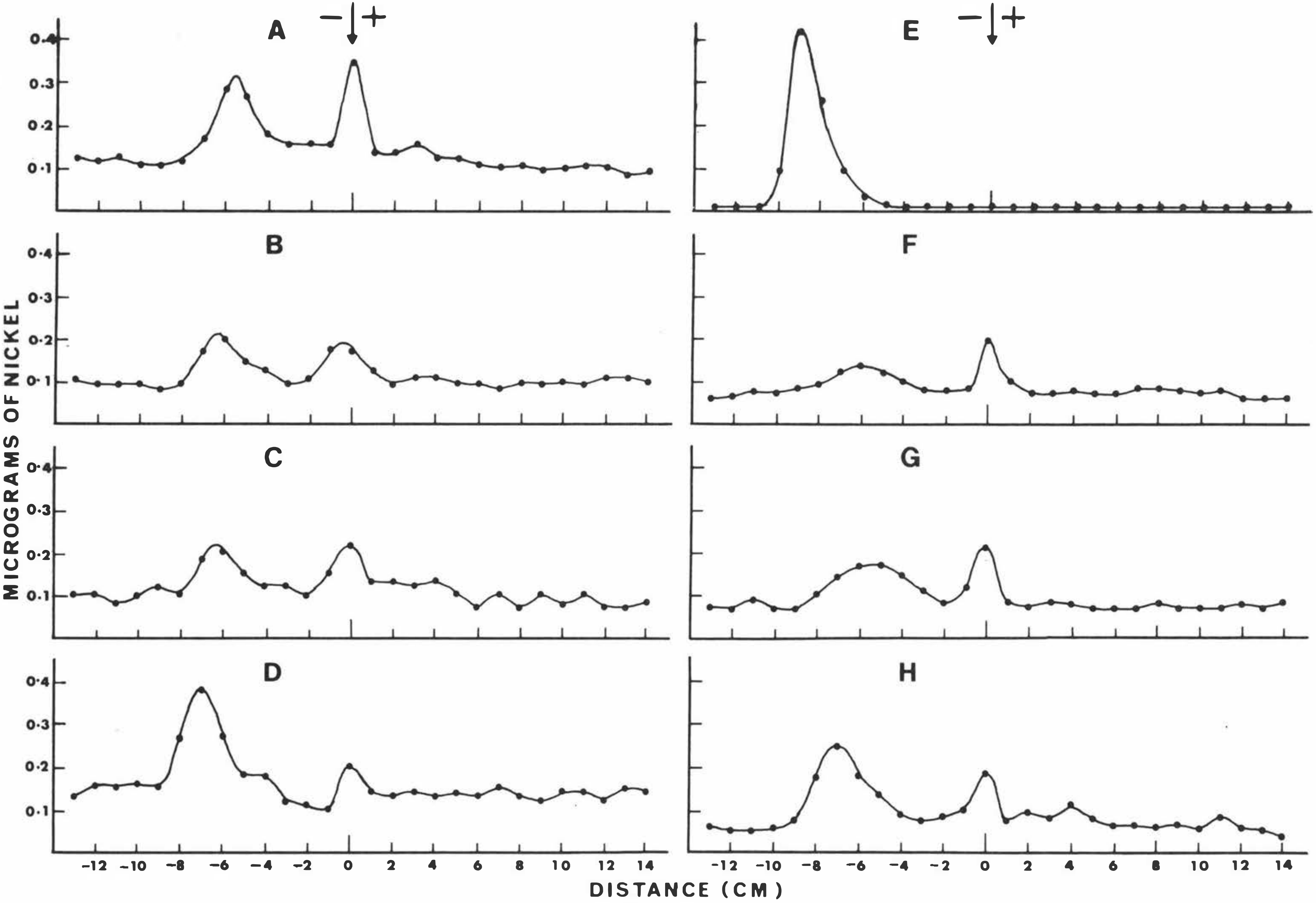

A
E
B
F
C
G
D
H
− +
− +
0·4
0·3
0·2
0·1
MICROGRAMS OF NICKEL
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
DISTANCE (CM)

Figure V-3

4. Discussion.

The variable ratio of mobile to immobile nickel observed when different quantities of extract were loaded onto the paper, suggested that only one form of nickel compound was present and that the nickel on the origin was retarded by the large quantities of uncharged compounds present in the extract. This possibility was supported by later work.

Since the nickel in the extract did not migrate as far as the free divalent cation, it seemed that the nickel compound had a positive charge less than 2. A likely possibility to satisfy this criterion would be an amino acid chelate with nickel, in which only one ligand was coordinated through the carboxylic oxygen and the amino nitrogen.

Monk (1951) showed that at pH $\approx$ 5.0 glycine formed a 1:1 complex with nickel ($pK_1 \approx 6.0$) and at pH $\approx$ 6.0 a 2:1 complex was formed ($pK_2 \approx 5.0$). A similar observation was made for alanine for which the appropriate constants were $pK_1 \approx 6.0$ at pH $\approx$ 5.5 and $pK_2 \approx 4.7$ at pH $\approx$ 6.5. Katzin and Gulyas, (1969) showed that in mole ratios to nickel of one, the amino acids, alanine, serine, valine, arginine, ornithine and glutamic acid gave 1:1 complexes with nickel by coordinating through the carboxyl and α-amino groups. With mole ratios greater than one, evidence for the bis-chelate was apparent at approximately pH 7.0. Datta <u>et al.</u>, (1959) also considered that coordination to the nickel ion was through the carboxyl and α-amino groups of α-amino acids. These observations suggested that a chelate of nickel with an amino acid could give rise to the results observed in the present study. However, this hypothesis was tentative as there were likely to be other ligands in the leaf tissue which could give similarly charged chelates.

F. INVESTIGATIONS OF THE SOLUBLE METAL COMPLEXES IN THE FRESH LEAVES OF Q.ACUTIFOLIA.

1. Introduction.

Further studies on the soluble forms of nickel in the leaves of *Q.acutifolia* were undertaken. Since approximately 90% of the total nickel was present in the supernatant fraction after differential centrifugation, this fraction was chosen for study preferentially to the 80% aqueous ethanol extract of the freeze-dried leaves which contained only 60 to 80% of the total nickel.

The extract used in this study was prepared by blending the fresh leaves in distilled water, straining, and centrifuging at 20,000g for 30 minutes. Analyses of the two fractions obtained were the same as for the corresponding fractions obtained in the differential centrifugation experiments, analyses of which are shown in Table V - 2.

2. Electrophoresis of the extract.

The fraction obtained above was concentrated and subjected to electrophoresis under the usual conditions.

(a) Results.

The resulting electrophoretogram was analysed for nickel, copper, zinc and iron and the results are shown in Figure V - 4. The appropriate cation standards are also shown. As was observed previously for the freeze-dried leaf tissues, nickel was present as both a cation and an immobile form. Copper, zinc and iron were all present in the extract as anionic complexes. No cations of zinc or copper were apparent. In addition to the anionic complexes of iron and zinc, immobile forms were also evident.

The electrophoretogram was viewed under ultraviolet light (350nm) and sprayed with a ninhydrin / cadmium acetate reagent (acetone / water / acetic acid / ninhydrin / cadmium acetate, 89 / 8 / 2 / 1 / 0.05). Both ultraviolet-fluorescent and ninhydrin-positive compounds were evident in the same positions as the copper, zinc and iron complexes.

It was also observed that the copper standard did not migrate as far as either the nickel or the zinc standards.

(b) Discussion.

(i) Nickel.

It was apparent from these findings that the nickel complex extracted from the fresh leaves of Q.acutifolia was of a similar charge to that extracted from the freeze-dried leaves, although there was no evidence to indicate that the ligands coordinated to the nickel ion in each case were the same. However, unless a ligand with a stronger affinity for nickel occurred in the cell organelles and consequently was present in the freeze-dried tissue but not in the supernatant fraction, there was a high probability that the ligand was the same in both extracts.

Figure V - 4 Patterns of some metals after electrophoresis at pH 5.3 of the aqueous extract of fresh *Q.acutifolia* leaves.

●	Metal in the extract
○	Cation standard
A	Nickel
B	Zinc
C	Copper
D	Iron
E	Standard iron-citrate complex

N.B. Three separate papers were used:

(1) A , D

(2) B , C

(3) E

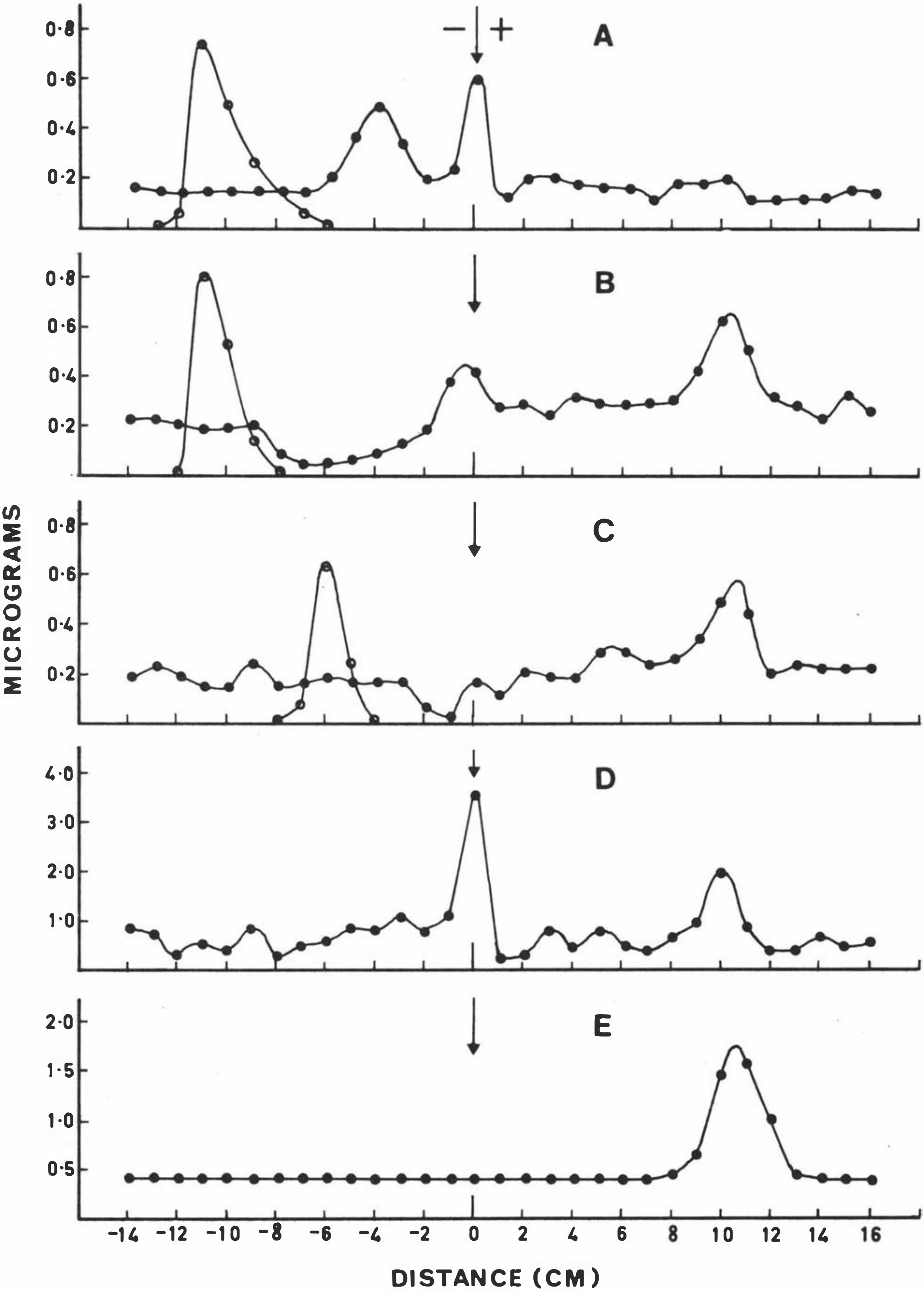

A
B
C
D
E
− +
MICROGRAMS
0·8
0·6
0·4
0·2
4·0
3·0
2·0
1·0
2·0
1·5
1·0
0·5
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
DISTANCE (CM)

Figure V-4

(ii) Copper, zinc and iron.

The occurrence of soluble copper and zinc complexes in plant tissues is well known. Bowen *et al.* (1962) showed that in the aqueous ethanol extract of tomato plants, two forms of zinc were present; one mobile and one immobile on paper chromatography with 95% aqueous ethanol. Peterson (1969) observed two anionic zinc complexes in the 80% aqueous ethanol shoot extract of a zinc-tolerant species *Agrostis tenuis*. Bremner and Knight (1970) working with ryegrass found evidence for three anionic zinc complexes and two anionic copper complexes in the 80% aqueous ethanol extract of this plant. The copper accumulating plant *Becium homblei* from Rhodesia was shown to contain copper complexes apparently with amino acids, in the water extract of its leaf tissue. (Reilly *et al.*, 1970)

In the present study, only one anionic complex of each metal, copper and zinc, was observed and both these complexes had the same charge. No cations of either metal were evident and this finding agrees with other work. (Bremner and Knight, 1970; Peterson, 1969; Reilly *et al.*, 1970)

Only a small portion of the total iron in the leaf tissues was extractable and this was shown by electrophoresis to be as an anionic complex. The existence of complexes of iron in plant tissues is made necessary by the fact that the ferric cation precipitates as the hydroxide at pH values greater than about 3. It was therefore, not surprising that an iron complex was observed both in the present study and in the xylem exudates studied by other workers. (Schmid and Gerloff, 1961; Tiffin, 1966a; 1966b; Tiffin and Brown, 1962).

Tiffin and Brown (1962) suggested that iron in the exudate of soya beans was coordinated to both malic acid and malonic acid, but in later work, (Tiffin, 1966a, 1966b) the author suggested that the ligand in soya bean, sunflower, tomato and cucumber exudates was citrate. This evidence was based firstly on the similar electrophoretic mobilities of the natural complex and an artificial complex of iron and citrate, and secondly on the presence of citrate in the iron-containing spot on the electrophoretogram of the exudate.

3. Comparisons with chelates of citric acid and oxalic acid.

(a) Introduction.

In view of previous work by Tiffin (1966a, 1966b) in which the presence of an iron citrate complex in the exudate from various plants was postulated and the work by Lyon *et al*. (1969a, 1969b) in which the trisoxalatochromium (III) complex was shown to exist in the tissue of *Leptospermum scoparium*, it was decided to investigate the chelates formed between nickel, copper, zinc and iron and oxalic and citric acids.

(b) Method.

Solutions containing (a) 0.05 M citric acid and (b) 0.05 M oxalic acid were made 0.005 M with respect to each of the four cations nickel, copper, zinc and iron. These two solutions as well as standards of each metal were electrophoresed and the electrophoretogram dried and analysed.

(c) Results.

For nickel, copper and zinc in both citric and oxalic acids, only positive compounds were present on the electrophoretogram, whereas for iron an anionic complex with citric acid was formed which had a similar mobility to the iron complex present in the extract (Figure V - 4E). No complex of iron with oxalic acid was formed.

(d) Conclusions.

From these observations it was concluded that the naturally-occurring complexes of nickel, copper and zinc documented in this thesis were unlikely to contain either oxalate or citrate ions. On the other hand, it was shown that the natural complex of iron could be a citrate complex as suggested by Tiffin (1966a, 1966b) for plant exudates.

4. Chromatography of the copper, zinc and iron complexes.

(a) Introduction.

It was observed in part 3 above that the complexes of copper, zinc and iron all occurred in identical positions on the electrophoretograms and that ultraviolet-fluorescent and ninhydrin-positive compounds also occurred in the same place. To separate the compounds present with the complexes, paper chromatography was resorted to.

(b) Method.

Four electrophoretograms of the extract were run and 2cm wide strips containing the copper, zinc and iron complexes were eluted with water. The eluate was concentrated under reduced pressure and applied as two 2cm long streaks, 20cm apart, to a sheet of Whatman 3 MM chromatography paper.

This paper was developed by descending chromatography with a butanol/ acetic acid/ water (12:3:5) solvent.

After the solvent front had moved approximately 40cm, the paper was dried and the positions of both the U.V. - fluorescent and ninhydrin-positive compounds were recorded.

The duplicate chromatograms were analysed for copper, zinc and iron by the same procedure described earlier for the electrophoretograms.

(c) Results.

The analyses of the two chromatograms were averaged and the resulting scan is shown in Figure V - 5. The

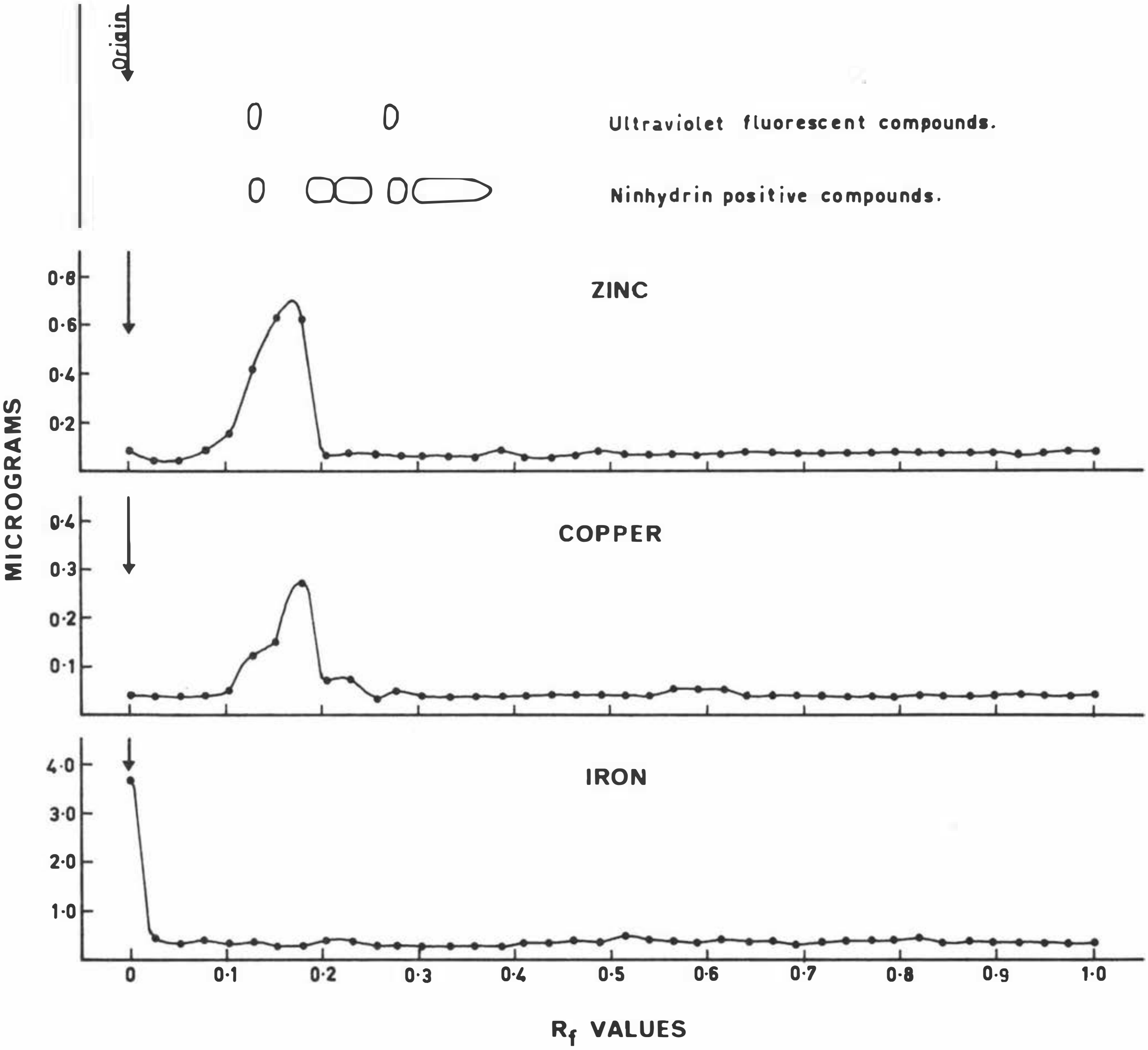


Figure V-5. Chromatography in butanol/acetic acid/water 12:3:5 of metal complexes isolated by preparative scale electrophoresis.

positions of the compounds located by ninhydrin and U.V. light are also shown.

Although poor resolution of the metal complexes was obtained, the results indicated firstly, that at least seven compounds were present in the 2cm strips eluted off the electrophoretograms, secondly that the metal complexes did not apparently correspond to any of the compounds visible on the chromatogram and thirdly that the copper and zinc complexes were very similar to each other with respect to their mobilities in the solvent used.

The iron complex did not move from the origin.

(d) Discussion.

The similar behaviour of the copper and zinc complexes in both the electrophoresis and the chromatography systems was in agreement with the observations of Bremner and Knight (1970) for ryegrass. These authors showed that on electrophoresis at pH 7.5, both the copper and zinc complexes had similar mobilities, although at pH 5.3 the zinc complex was slightly slower than the copper complex. In addition, the elution volumes of both complexes from a Sephadex G - 15 column were very similar.

The significance of the apparent similarities between the copper and zinc complexes cannot be fully assessed until the structures of the complexes are determined and the mechanisms of formation are elucidated.

5. Partial purification of the extract by Gel Filtration.

(a) Introduction.

Preparative-scale electrophoresis as used for the copper, zinc and iron complexes above, was found to be unsuitable for nickel because large amounts of other compounds occurred in the same place as nickel on the electrophoretogram.

Procedures to purify the extract by cation exchange using Dowex 50 - X 8 (Wang, 1960), by column chromatography on cellulose and by thin layer chromatography on silica gel all proved to be unsuitable.

Gel filtration on Sephadex, because of the mild conditions under which separation is achieved, was the most attractive method to study in detail. Separation by these gels depends primarily on molecular size and since the nickel complex in the extract was suspected to have a low molecular weight i.e. <500, Sephadex G - 10 with a working range from molecular weight 200 to 700 was the obvious choice. Unfortunately, this grade of gel was not available at the time and Sephadex G - 25 - 300 was used. It was found, however, that satisfactory results were obtained with this gel.

(b) Procedure.

The gel was allowed to swell in distilled water for 24 hours before packing to produce a bed 11cm x 1cm. This bed was eluted with 0.1 M hydrochloric acid until the eluate was free from metal ions (by atomic absorption analysis) and then washed with distilled water until the eluate was pH $\approx$ 5.

Half ml of the concentrated extract (containing 9.0µg nickel) was applied to the column and eluted with distilled water. Ten drop fractions (0.8ml) were collected. The absorbance of each solution was measured at 550nm and each solution was analysed directly for nickel, copper and zinc. The results are shown in Figure V - 6A. A second column of the same dimensions was prepared and the same volume of extract was passed through the column. From the colours of the fractions it was apparent that the bed volumes of both columns were almost identical.

The fractions between the two arrows shown in Figure V - 6A were combined and concentrated. This sample, which was estimated to contain approximately 6.5µg of nickel, was applied to a third column of similar dimensions to the previous two and eluted with water. The fractions were analysed for nickel, copper and zinc and the results are shown in Figure V - 6B.

An approximate estimation of the purification obtained was made by repeating the procedure described in the preceding paragraph. The same fractions were combined and this sample as well as 0.5ml of the original extract were dried under vacuum over silica gel.

Both the raw extract and the eluate from the column were subjected to electrophoresis and the scans of the electrophoretograms are shown in Figure V - 6 C and D.

Figure V - 6 Gel filtration of the aqueous extract of fresh *Q.acutifolia* leaves.

A First column

B Second column

C Pattern of nickel after electrophoresis at pH 5.3 of the aqueous extract.

D Pattern of nickel after electrophoresis at pH 5.3 of the partially purified extract from the first column.

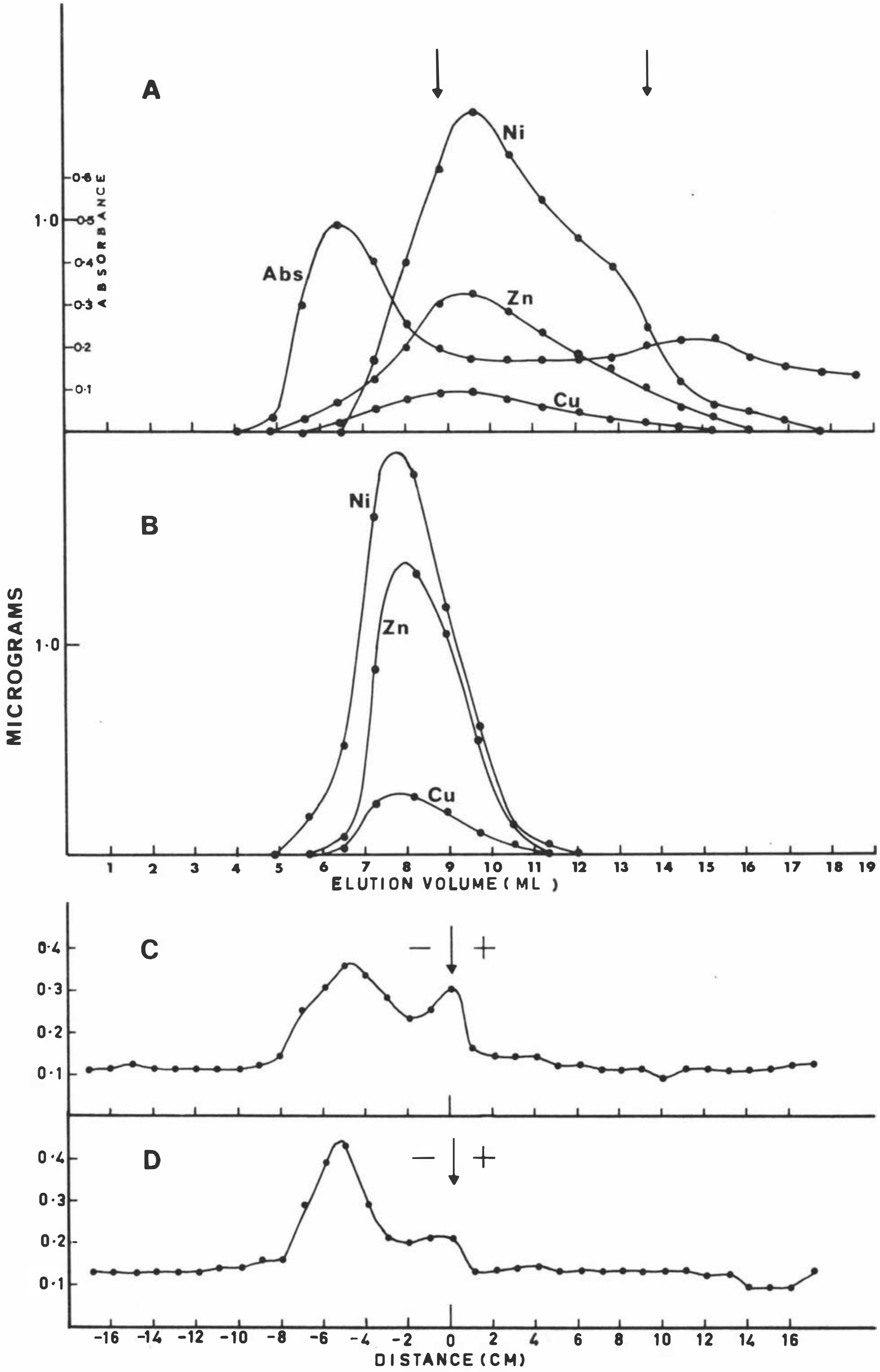
A
B
C
D
Ni
Zn
Cu
Abs
ABSORBANCE
0·1
0·2
0·3
0·4
0·5
0·6
1·0
MICROGRAMS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
ELUTION VOLUME (ML)
0·1
0·2
0·3
0·4
− +
-16 -14 -12 -10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16
DISTANCE (CM)

Figure V-6

(c) Results.

From the results presented in Figure V - 6A, it can be seen that all three metal complexes had similar elution volumes.

From the dry weights of the fractions collected between the arrows shown on Figure V - 6 and of the 0.5ml of the raw extract, it was found that the fraction collected contained 4% of the material applied to the column. This sample also contained 75% of the nickel in the original volume of extract applied to the column.

When this fraction was applied to a second column, better resolution was obtained (Figure V - 6B) but the extra purification gained was less than 2% (relative to the original extract) since most of the impurities were eluted with the metals. Recovery of nickel from this column was greater than 90%.

High-voltage electrophoresis of the raw extract and the partially purified extract (Figures V - 6C and D) showed that a nickel complex of similar charge existed in both and that in the partially purified extract, no nickel remained on the origin.

(d) Conclusions.

A separation using one column was adopted as standard procedure for the partial purification of the nickel complex. Although the purification was far from complete (the ratio of nickel to impurities was still approximately 1:2000) the resulting extract was suitable for use in subsequent studies.

6. High-voltage electrophoresis of the partially purified extract.

(a) Introduction.

Since nickel is apparently non-essential to plant nutrition at the levels being studied, it is likely that this metal is accumulated into plant tissues by passive rather than active processes, unless it is accumulated in place of a small portion of some other metal. If the processes are passive then from the ligands available, the most stable nickel chelate will be formed. Under these circumstances there would probably be an excess of free ligand present in the plant cells since the manufacture of the ligand would not be metabolically regulated by the nickel concentration in the plant, nor would the nickel concentration in the plant be determined by the availability of the ligand. To test this hypothesis the following experiment was carried out.

(b) Procedure.

Three 50μl aliquots of the partially purified extract, each containing 0.5 to 1μg of nickel, were mixed with different amounts of the divalent nickel cation ($NiCl_2$ in water) in three experiments (a) 1μg (b) 5μg and (c) 10μg. After mixing, the extracts plus the added nickel was subjected to electrophoresis. The analyses of the papers are shown in Figure V - 7. A scan of the extract without nickel added is also shown.

(c) Results.

If the above hypothesis was incorrect, then two peaks were anticipated, one corresponding to the nickel complex, the other to the faster moving divalent nickel ion. On the other hand, if the hypothesis was correct, only one peak containing both the natural and artificial complex was expected.

As can be seen from Figure V - 7 A, B, C, D, only one peak was observed, but as the added amount of nickel was increased the peak moved further.

The reasons for these observations were not apparent and an experiment was performed in which different amounts of the divalent nickel cation in distilled water were electrophoresed. (Figure V - 7, E, F, G) The amounts applied to the papers are given as loadings (μg of nickel/cm) for both the extracts and the standard nickel solutions. These values are shown in the key to Figure V - 7. It can be seen from the scans of the divalent cation (Figure V - 7 E,F,G) that a similar dependence of migration distance on loading was observed as for the extract mixtures.

(d) Discussion.

From Figure V - 7 it can be seen that when the ratio of added nickel was increased up to 10:1 and 20:1 (Figures V - 7 C and D) only one peak was evident, but this may have been due to the poor resolution of the analytical method since the contribution of nickel from the extract lay within the total

Figure V - 7 Patterns of nickel after electrophoresis at pH 5.3 of both the partially purified aqueous extract from fresh <u>Q.acutifolia</u> leaves and nickel cation standards.

A	Extract	(0.0200 μg/cm)
B	Extract + 1 μg Ni^{2+}	(0.0410 μg/cm)
C	Extract + 5 μg Ni^{2+}	(0.156 μg/cm)
D	Extract + 10 μg Ni^{2+}	(0.254 μg/cm)
E	Ni^{2+}	(0.0126 μg/cm)
F	Ni^{2+}	(0.0261 μg/cm)
G	Ni^{2+}	(0.314 μg/cm)
H	Ni^{2+} in NaOH/acetic acid buffer pH 5.3	(0.0110 μg/cm)

N.B. The same quantity of extract was used in A, B, C and D.

Figures in parenthesis are the loadings of nickel applied to the paper.

The peak corresponding to the nickel in the extract (A) is reproduced on B, C and D at the appropriate scale for each pattern.

Separate papers were used for each electrophoretogram.

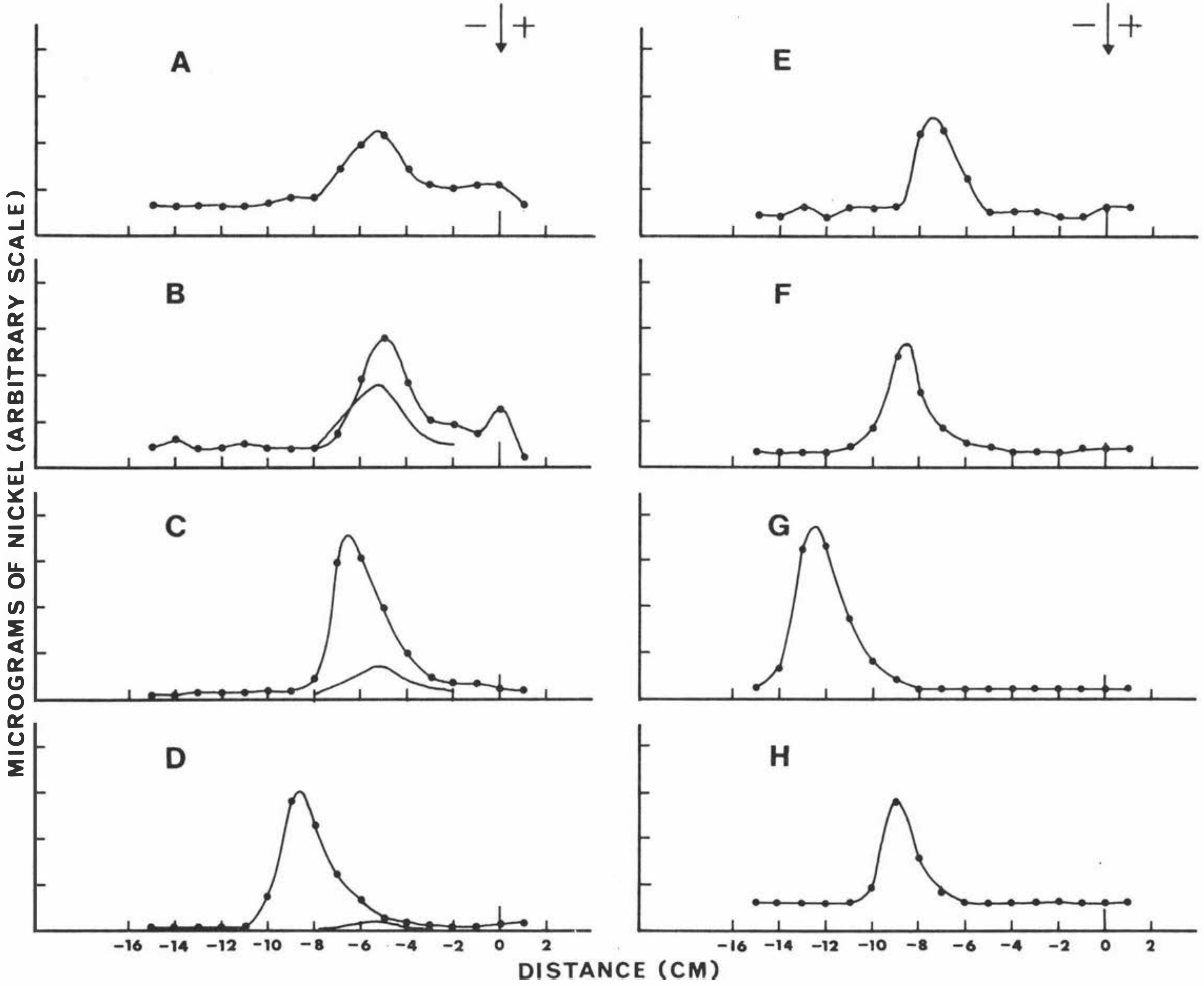

A
B
C
D
E
F
G
H
− +
− +
MICROGRAMS OF NICKEL (ARBITRARY SCALE)
-16 -14 -12 -10 -8 -6 -4 -2 0 2
-16 -14 -12 -10 -8 -6 -4 -2 0 2
DISTANCE (CM)

Figure V-7

peak observed. (see Figure V - 7, A,B,C and D). If this was the case then the major peaks should have been in the normal position of the divalent nickel cation i.e. at approximately -12cm. This, however, was not observed.

If pyridine from the buffer was coordinating to the nickel ions then, although the charge remained the same, the mass would increase and a decrease in migration distance would be observed. When, however, the divalent nickel cation was electrophoresed in a sodium hydroxide / acetic acid buffer (pH 5.3), a similar retention of the nickel was observed which indicated that pyridine was not causing the retention.(Figure V - 7E)

An alternative explanation is that a small amount of ion exchange occurred between the cations and the phenolic and carboxylic acid groups in the paper.

Pickering (1960) observed that on Whatman No.1 paper the cations lead, zinc, cadmium, copper and nickel were absorbed. This author considered the mechanism to be ion exchange and showed that the exchange capacity of the paper was approximately 4 μg / gm. Frew and Pickering (1970) studied the absorption of the copper (II) ion onto Whatman No.41 paper and explained the effect by Donnon membrane theory.

In view of the findings of these authors it seems likely that during the electromigration of cations through paper, there are two processes in competition; a relatively labile ion exchange and the migration of the cations in the electric field. Where the concentration of cations on the paper is small, the effects of ion exchange are significant and the major part of the cations are retarded. However, when high concentrations of cations are applied to the paper, most of the cations move under the influence of the electric field while a small amount of the cations are retarded by ion exchange, resulting in an asymmetrical peak.

Further support for the above postulate was that the anionic complexes in the extracts showed no retention, regardless of the loading. In every electrophoretogram analysed for copper, zinc and iron, these metals all occurred in the same position ± 0.5cm. Also these anionic complexes generally showed higher resolution on the electrophoresis papers than did the nickel complexes.

Although the above results seemed to indicate that an excess of the nickel chelate ligand was present in the extract, because of the poor resolution of the divalent cation from the complex the results were considered inconclusive. Resort was made to paper chromatography to obtain a better separation of the divalent cation from the complex.

7. Paper chromatography of the partially purified extract.

(a) Different solvent systems.

(i) Method.

Chromatography papers were prepared by applying the partially purified extract (50µl to 100µl) containing between 0.5µg and 1.0µg of nickel, and approximately the same amount of divalent nickel cation in distilled water as two separate streaks onto Whatman 3 MM chromatography paper.

The papers were developed a distance of 30cm to 40cm by descending chromatography with the following solvents:

Pyridine / acetic acid / water	10 / 4 / 986 v/v
Butanol / Ethanol / water	2 / 2 / 1 v/v
Butanol / acetic acid / water	12 / 3 / 5 v/v
Dioxane / water	8 / 2 v/v
Methylethyl ketone / acetone / water	3 / 1 / 0.6 v/v
Ethanol / water	8 / 2 v/v

After drying, the chromatograms were analysed as described previously; extracts and standards being analysed separately.

(ii) Results.

The scans of each chromatogram are shown in Figure V - 8. Because of the uneven movement of the solvent in some cases, all distances were converted to R_f values. The extract and standard scans from the same paper are shown on the same figure.

The best resolution of the nickel complex from the divalent nickel cation was obtained with the ethanol / water solvent.

(b) Ethanol / water solvent.

(i) Method.

Two aliquots of the partially

Figure IV - 8 Chromatography of the partially purified extract from fresh *Q.acutifolia* leaves. Patterns of nickel.

○ nickel in extract
● Ni^{2+} standard
A ethanol/water 8/2
B dioxane/water 8/2
C butanol/acetic acid/water 12/3/5
D pyridine/acetic acid/water 10/4/986
E methylethylketone/acetone/water 15/5/3
F butanol/ethanol/water 2/2/1

N.B. Standard and extract run on the same paper.

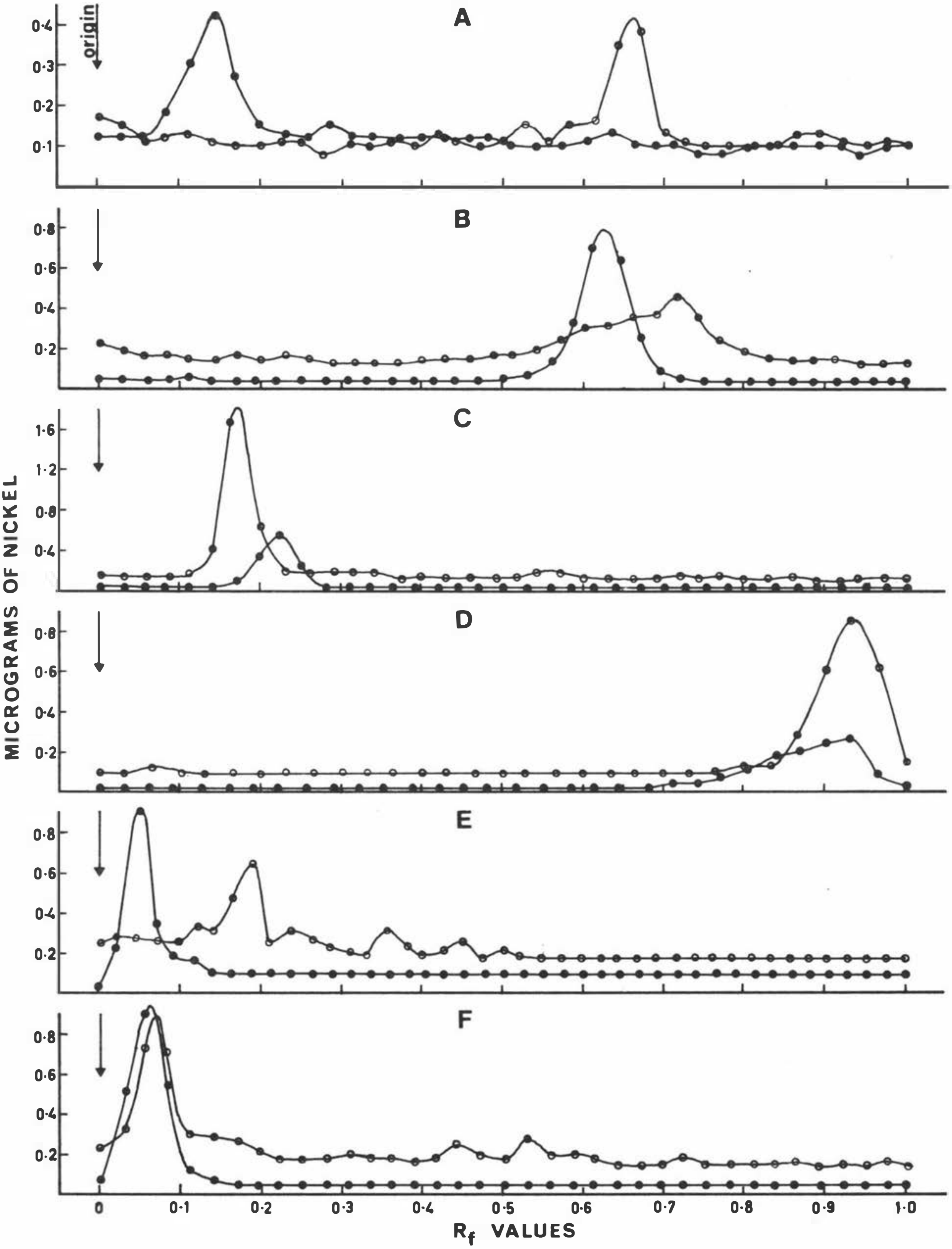

A
B
C
D
E
F
origin
MICROGRAMS OF NICKEL
0·1
0·2
0·3
0·4
0·6
0·8
1·2
1·6
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
R$_f$ VALUES

Figure V-8

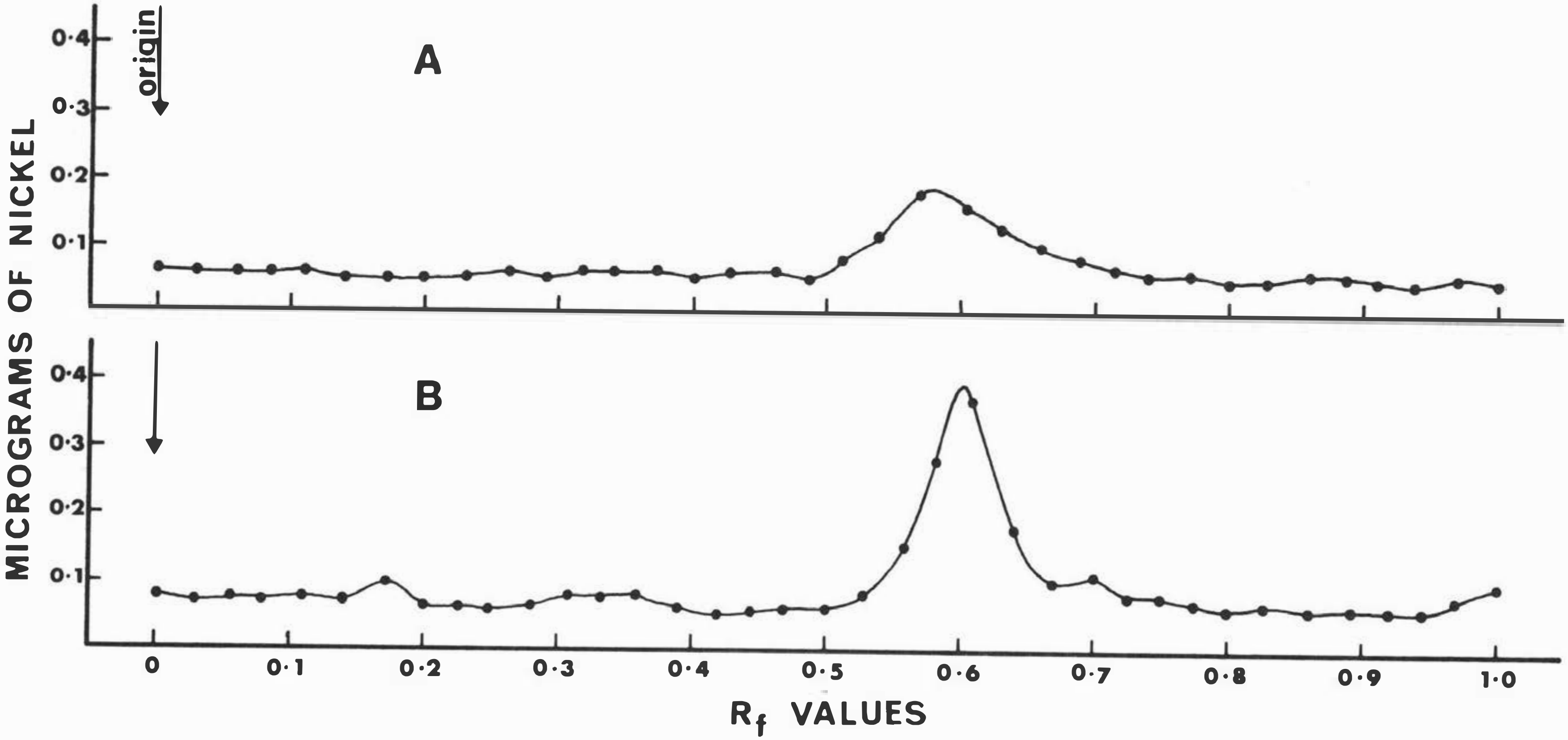


Figure V-9. Chromatography in ethanol / water 8:2 of the Q. acutifolia leaf extract.

A. extract

B. extract + Ni^{2+}

purified extract were taken, each containing approximately 0.5 μg of nickel. To one of the aliquots, 0.5 μg of divalent nickel cation was added, and after mixing, this solution as well as the untreated aliquot were applied as two streaks to one sheet of Whatman 3 MM chromatography paper. This paper was developed with the ethanol/water solvent, dried, and each chromatogram analysed.

(ii) Results.

The scans of the two chromatograms are shown in Figure V - 9. All the nickel on each chromatogram moved as a single peak and both peaks showed similar R_f values.

(iii) Conclusions.

The above results showed that there were free ligands in the extract able to chelate to the added nickel. Whether the artificially formed chelate was the same as the naturally-occurring chelate was not possible to ascertain from these results but in view of their similar mobilities in this solvent and on electrophoresis (Figure V - 9B), it is possible that they were the same.

Conclusive evidence of this, however, must await future work.

G. CONCLUSIONS.

The results presented in this section showed that in all the species studied, the major part of the total nickel in the leaf tissues was soluble in 80% aqueous ethanol and water and also occurred in the supernatant fraction after differential centrifugation. It was also shown that the soluble nickel existed as a positively charged complex in all the species and that in Q.acutifolia, a complex(s) of the same charge and chromatographic mobility was formed when divalent nickel cations were added to the supernatant fraction. A smaller portion of the total nickel in all the leaf samples remained attached to the residue after solvent extraction and a similar portion, possibly the same portion, was shown to be associated with either the cell walls or the chloroplasts.

On the basis of these findings it was concluded that most of the nickel in the leaves of the species studied was present outside the cell organelles, either in the cytoplasm or the vacuole.

These observations were in contrast to those for copper and zinc. Both these metals were shown to be present in approximately equal amounts in the cell walls or chloroplasts and the supernatant fraction. Iron, on the other hand, was found to exist predominantly in the cell walls or chloroplasts. In the supernatant fraction of Q.acutifolia leaves, the positive complex of nickel was in contrast to the negatively charged complexes of copper, zinc and iron. The anionic complexes of copper and zinc were shown to be electrophoretically and chromatographically similar. It was also shown that the anionic complex of iron could be a chelate with citrate ions.

If the marked similarities observed in this study between the locations and forms of nickel in the different species can be extended to crops, then the results could have significance for human and animal nutrition. As nickel has been suggested as an essential element for some animals (Nielsen, 1970), although its importance for human nutrition has yet to be assessed, the occurrence of nickel in leaves predominantly in a soluble form, indicates that it may be lost from leaves by, say, boiling. In areas with low soil nickel and hense low plant nickel, such a loss could be of profound dietary significance. Alternatively, in areas with high concentrations of nickel in the soil, boiling the tissues may decrease the total nickel to an acceptable level.

If, in the future, nickel is established as an essential element for human nutrition, then these observations may be of considerable importance.

SECTION VI

SUMMARY AND GENERAL CONCLUSIONS

The findings of this thesis can be grouped into four categories, which although superficially different, are all fundamentally related to the general study of metals in our environment. These categories are:

(a) Sampling and analytical error associated with geochemical and biogeochemical data.

(b) Biogeochemical prospecting for nickel and copper.

(c) The biogeochemistry of calcium, magnesium, potassium and trace metals.

(d) The plant chemistry of nickel, copper, zinc and iron.

The problems of sampling and analytical error were considered in detail in SECTIONS II and III because of the importance of this error to the validity of the results obtained from statistical evaluations of the analytical data. It was observed that not only did the methods of sampling result in a significant error but that the techniques involved in sample preparation and analysis also contributed to the overall error in the data. These findings emphasised the need for statistical methods capable of separating the data into components due to environmental processes and components due to sampling and analytical error. It was shown that trend analysis was a suitable technique to achieve this separation.

The biogeochemical method of prospecting for nickel and copper was investigated and it was concluded that the method could be used to prospect for nickel by the analysis of the leaves of Nothofagus fusca and/ or Nothofagus truncata. It was also observed that the accumulations of most metals were similar for these two species and in view of the fact that these species were genetically similar to each other and to *N.cliffortioides* and *N.solandri*, it was suggested that the latter two species may give biogeochemical prospecting results similar to those for *N.fusca* and *N.truncata*. If future work shows these suggestions to be correct, then prospecting for nickel using the *Nothofagus* genus could be extended from the present altitude limit of approximately 3500 feet for *N.fusca* and *N.truncata* to above 4000 feet using *N.cliffortioides*.

Multiple regression analysis was used to improve the accuracy with which the concentrations of nickel and copper in the soil could be predicted from the concentrations of these metals in the leaf ash of the *Nothofagus* genus. The regression equations derived, made allowances for the various biogeochemical factors, such as the concentrations of other metals in the plants, which influenced the accumulation of nickel and copper by the plants. It was concluded that the use of multiple regression analysis offered the most promising tool for prospecting for copper with the biogeochemical method.

The ratios of the copper concentrations in the leaves to the concentrations of some other metals in the leaves, such as the copper/zinc ratio suggested by Warren and Delavault (1949), were investigated as possible indicators of the soils containing high concentrations of copper. The reliability, however, was found to be too low to make these ratios useful for prospecting.

The essential nature of metals for plant nutrition was observed to be the main factor influencing biogeochemical prospecting results. It was shown that when the concentration of a metal in the leaf ash divided by the total concentration of the metal in the soil was plotted against the total concentration of the metal in the soil, the resulting graph gave a good indication of the requirement of the plant for the metal and also of the minimum soil concentration at which the biogeochemical method would be useful.

Observations were made of the biogeochemical characteristics of some metals. Consideration of the analytical data showed that different plant species distributed some metals in different ways between their leaves and twigs and that the concentrations of some metals were related to the area of the leaves. Interactions between metals in the leaves and between metals in the leaves and soil, were observed for the Nothofagus genus and although most of these were inexplicable, they indicated many features which warrant closer study.

Investigations of the relationships between the metal concentrations in the leaves and in the soils showed that, in general, the accumulation of metals essential to the growth of the plants was regulated by the plants themselves with little influence from the soil. An exception to this was copper, although the accumulation of this metal was observed to be influenced to only a small extent by the concentrations of copper in the soil.

In the final part of this thesis, the quantities and chemical forms of some trace metals in the extracts of leaves of different trees were studied. The use of atomic absorption spectrophometry to detect small amounts of metals separated from plant extracts on chromatography and electrophoresis papers was demonstrated. These studies suggested that nickel was distributed differently in the leaves from copper, zinc and iron and that the soluble portions of these latter three metals were present as anionic complexes in contrast to a cationic complex of nickel.

In conclusion, this thesis has ranged over many disciplines; from analytical chemistry to geochemistry, biochemistry and statistics. The results reported have indicated promising avenues of future research. In particular, *N.fusca* and *N.truncata* should now be applied to the search for nickel in New Zealand and an evaluation of the usefulness of *N.cliffortioides* and *N.solandri* should be made. Interactions between metals in plants and soils were observed which warrant further investigation under controlled conditions and the application to biological studies of instrumental methods of analysing microgram amounts of metals, offers a challenging field of research.

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APPENDIX I.

Plant species recorded.

The following is a list of the plant species recorded in the plant mapping survey (SECTION III) and includes both trees and shrubs. In general, ferns and other small plants were not considered except for the fern *Blechnum discolor* which was included because of its wide distribution. For each line transect, eleven 400ft^2 quadrats were studied and for both transects the total number of each species is given below as well as the number of each species times the mean trunk diameter for that species. This latter value gives an approximate indication of the relative contribution of each species to the vegetation cover.

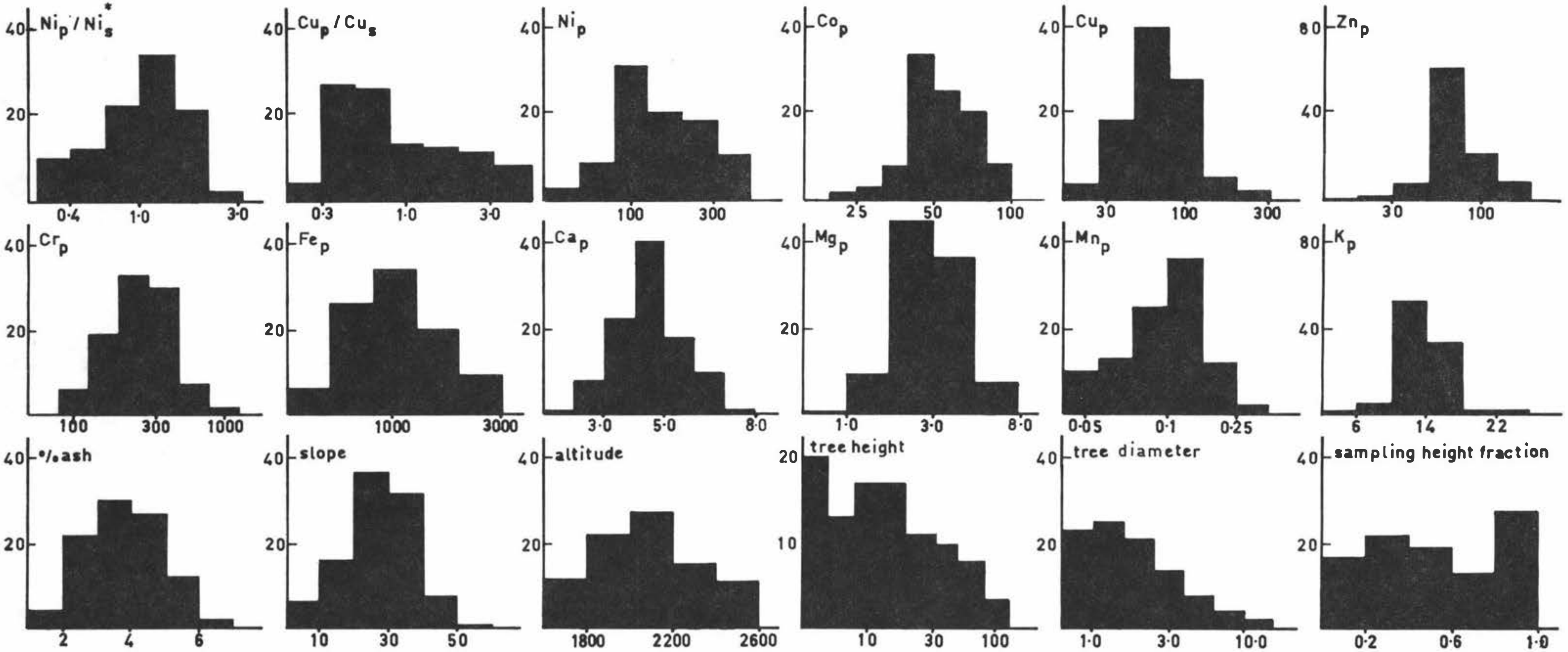
	(2,0) - (2,20)		(7,0) - (7,20)	
	A	B	A	B
Blechnum discolor (Forst.f.) Keys	122	-	117	-
Carpodetus serratus J.R. et G. Forst	2	6	6	6
+*Coprosma banksii* Petrie) *C. linariifolia* Hook f.) *C. foetidissima* J.R. et G. Forst)	83	51	21	17
Cyathodes fasciculata (Forst.f.)	11	3	7	3
Elaeocarpus hookerianus Raoul	1	1	0	0
Griselinia littoralis Raoul	3	20	2	5
Metrosideros umbellata Cav.	14	17	3	33
Neopanax anomalum (Hook.) Allan	2	2	0	0
N. simplex (Forst.f.) Allan	0	0	1	1
Nothofagus fusca (Hook.f.) Oerst	30	272	9	81
N. truncata (Col.) Ckn	41	57	43	103
N. menziesii (Hook.f.) Oerst	24	47	54	112
Podocarpus ferrugineus G.Benn ex D.Don	1	1	1	0.5
P. hallii Kirk	2	14	7	28
Pseudopanax crassifolium (Sol. ex A.Cunn.) C. Koch	6	5	9	26
Pseudowintera colorata (Raoul) Dandy	8	8	9	17
Quintinia acutifolia Kirk	64	70	91	155
Weinmannia racemosa Linn.f.	20	124	47	237

Identifications were made by the Department of Botany, Massey University. Nomenclature after Allan, H.H., 1961, Flora of New Zealand, Vol. 1, Wellington, R.E. Owen, Govt. Printer.

[+] Hybridisation between species of the *Coprosma* genus is common where they occur together and the samples submitted for identification did not allow exact distinctions to be made.

A No. of specimens recorded

B No. of specimens x mean trunk diameter (inches)



Appendix II. Frequency distributions of variables used in multiple regression analysis.

* p=plant , s = soil

APPENDIX III.

Correlation coefficient programme.

A. Description of the programme.

The programme listed in APPENDIX III B although written specifically for the applications described in this thesis, is readily adaptable to other sets of data.

All the data for a particular sample e.g. 10 metal concentrations, the percentage ash, and the site number for a plant sample, are punched onto one card with each variable occupying the same columns on each data card. In preparation for the computation of the correlation coefficients, a data matrix is read into disk storage. This matrix may contain up to 13 variables although only the first 12 are correlated. The number of samples read in, is limited by the amount of disk storage available. The variables placed in the matrix may be read from one or two data decks as follows:

(1) Up to 13 variables may be read from either the plant data deck or the soil data deck.

(2) Up to 6 variables from the plant data deck and up to 5 variables from the soil data deck may be read. If less variables are used, the number of soil variables must be one less than the number of plant variables.

All the variables are transformed to base 10 logarithms and correlation coefficients and the slopes of the reduced major axes are computed and printed out for all possible combinations of two variables.

Further options allow the plant variables in the matrix from (2) above to be replaced by the plant variable divided by the corresponding soil variable (i.e. relative accumulation). In addition, all the metal concentrations in the plants may be converted from an ash weight basis to a dry weight basis. A printout of the data may also be made. Instructions for the implementation of these options, as well as for the requirements of the control cards to precede the data are given at the head of the programme listing in APPENDIX III B.

APPENDIX IIIB

CORRELATION COEFFICIENT PROGRAMME
FORTRAN IID

```
    CORRELATION COEFFICIENTS,GEOMETRIC MEANS,STANDARD DEVIATIONS,
    REDUCED MAJOR AXES

    PROGRAMME ALLOWS DATA TO BE READ FROM ONE OR TWO DATA DECKS
    PLANT DATA IS FED IN AS ASH CONCENTRATION
    ALL INPUT DATA TRANSFORMED TO BASE 10 LOGARITHMS
    SENSE SWITCH 1 ON CONVERTS DATA TO DRY WEIGHT CONCENTRATION
    SENSE SWITCH 2 ON REPLACES ODD VARIABLES WITH ODD VARIABLES
    DIVIDED BY NEXT EVEN VARIABLES
    SENSE SWITCH 3 ON PRINTS OUT INPUT DATA
    SENSE SWITCH 4 OFF, READS NEW FORMAT CARDS BUT USES PREVIOUSLY
    READ IN DATA DECK
    SENSE SWITCH 4 ON REPEATS COMPUTATIONS WITH SAME DATA DECK
    SENSE SWITCH 4 OFF READS NEXT DATA DECK
    CARDS TO PRECEDE DATA DECK ARE AS FOLLOWS
    TITLE CARD . TITLE MAY OCCUPY UP TO 79COLUMNS
    READ FORMAT FOR FIRST DATA DECK
    READ FORMAT FOR SECOND DATA DECK.A FORMAT CARD MUST BE INSERTED
    IN THIS POSITION EVEN IF NO SECOND DATA DECK IS USED
    CARD CONTAINING VARIABLES KL,IJ,JK,IJK,K,N, IN FORMAT 6I5 WHERE
    KL=TOTAL NUMBER OF VARIABLES TO BE READ,KL MAX=13
    IJ=NUMBER OF VARIABLES TO BE CORRELATED,IJ MAX=12
    JK=NO. OF CORRELATION COEFFICIENTS TO BE COMPUTED,JK MAX=66
    IJK=COLUMN CONTAINING FRACTION ASH.IF NO ASH THEN IJK=1
    K=1,ALL VARIABLES ARE CHANGED TO DRY WEIGHT CONCENTRATION
    K=2, ODD VARIABLES ARE CHANGED TO DRY WEIGHT CONCENTRATION
    N=1, ONE DATA DECK TO BE READ
    N=2, TWO DATA DECKS TO BE READ
    A CARD CONTAINING A NUMERAL 9 IN COLUMN 1 MUST FOLLOW EACH
    DATA DECK
    MEANS ARE GEOMETRIC
    STANDARD DEVIATIONS IN UNITS OF LOGARITHM TO BASE TEN

    DIMENSION A(13),S(12),SQ(12),AV(12),GM(12),SD(12),B(12)
    DIMENSION SP(66),X(66),Y(66),Z(66),R(66),RMA(66),MD(12)
    DIMENSION IFOR(80),JFOR(80)
    DEFINE DISK(13,1000)
 10 READ 20
 20 FORMAT (80H
   1
 21 READ 22,IFOR
    READ 22,JFOR
```

```
   22 FORMAT (80A1)
      READ 23,KL,IJ,JK,IJK,K,N
   23 FORMAT (6I5)
      DO 11 I=1,12
   11 A(I)=0
      IDEX=1
   30 READ IFOR,M,(A(I),I=1,KL,N)
      IF(M-9)50,60,50
   50 RECORD(IDEX)A
      GO TO 30
   31 IDEX=1
   32 FETCH(IDEX)A
      IDEX=IDEX-2
      READ JFOR,M,(A(I),I=2,KL,N)
      IF(M-9)51,61,51
   51 RECORD(IDEX)A
      GO TO 32
   60 GO TO (61,31),N
   61 ISAVE=IDEX
   70 DO 80 I=1,IJ
      S(I)=0
      SQ(I)=0
   80 AV(I)=0
      DO 90 J=1,JK
   90 SP(J)=0
      AN=0.0
      IF(SENSE SWITCH 1)100,120
  100 PRINT 110
  110 FORMAT (25H DRY WEIGHT CONCENTRATION)
      GO TO 140
  120 PRINT 130
  130 FORMAT (21H CONCENTRATION IN ASH)
  140 IF(SENSE SWITCH 2)150,161
  150 PRINT 160
  160 FORMAT (7H RATIOS)
  161 PRINT 162
  162 FORMAT (//)
      PRINT 20
C
C     START OF MAIN LOOP
C
      IDEX=1
  170 FETCH(IDEX)A
      IF(IDEX-ISAVE)180,180,350
  180 IF(SENSE SWITCH 1)181,210
  181 JM=IJ-1
  190 DO 200 I=1,JM,K
C
C     FIRST OPTION MANIPULATION FOLLOWS
```

```
200 A(I)=A(I)*A(IJK)
210 IF(SENSE SWITCH 2)211,240
211 KJ=IJ-1
220 DO 230 I=1,KJ,2

    SECOND OPTION MANIPULATION FOLLOWS

    KK=I+1
230 A(I)=A(I)/A(KK)
240 IF(SENSE SWITCH 3)250,280
250 PRINT 270,(A(I),I=1,KL)
    DATA PRINTOUT FOLLOWS
270 FORMAT(13F10.4)
280 AN=AN+1.0
    DO 290 I=1,IJ
    B(I)=0.43429448*LOGF(A(I))
    S(I)=S(I)+B(I)
290 SQ(I)=SQ(I)+B(I)*B(I)
    J=0
    I=0
300 I=I+1
    JJ=IJ
310 J=J+1
    IF(J-JK)320,320,330
320 X(J)=B(I)*B(JJ)
    JJ=JJ-1
    IF(JJ-(I+1))300,310,310
330 DO 340 J=1,JK
340 SP(J)=SP(J)+X(J)
    GO TO 170
    END OF MAIN LOOP
    START OF MAIN CALCULATIONS
350 DO 360 I=1,IJ
    AV(I)=S(I)/AN
    GM(I)=EXPF(AV(I)/0.43429448)
360 SD(I)=SQRTF((SQ(I)-AN*AV(I)*AV(I))/(AN-1.0))
    J=0
    I=0
370 I=I+1
    KE=IJ
380 J=J+1
    IF(J-JK)390,390,400
390 Y(J)=AV(I)*AV(KE)
    Z(J)=(SQ(I)-AN*AV(I)**2)*(SQ(KE)-AN*AV(KE)**2)
    RMA(J)=SD(I)/SD(KE)
    KE=KE-1
    IF(KE-(I+1))370,380,380
400 DO 410 J=1,JK
```

```
410 R(J)=(SP(J)-AN*Y(J))/SQRTF(Z(J))

    END OF CALCULATIONS
    PRINT OUT OF RESULTS FOLLOWS

    PRINT 420
420 FORMAT (/16H GEOMETRIC MEANS)
    PRINT 430,(GM(I),I=1,IJ)
430 FORMAT (12F10.4)
    PRINT 440
440 FORMAT (/12H STD. DEVS. )
    PRINT 450,(SD(I),I=1,IJ)
450 FORMAT (12F10.4)
    PRINT 460,AN
460 FORMAT(/19H NUMBER OF SAMPLES=,F6.0/)
    PRINT 470
470 FORMAT(25H CORRELATION COEFFICIENTS/)
    KL=1
    DO 480 I=1,IJ
480 MD(I)=IJ+1-I
490 PRINT 500,(MD(I),I=1,IJ)
    KC=1
    MA=1
500 FORMAT (11H COLUMN NO.,12(2X,F4.0,4X))
    LA=IJ-2
510 NA=MA+LA
    PRINT 520,KC,(R(J),J=MA,NA)
520 FORMAT (4X,I2,4X,12F10.4)
    KC=KC+1
    LA=LA-1
    MA=NA+1
    IF(KC-IJ)510,530,530
530 GO TO (540,580),KL
540 PRINT 550
550 FORMAT (/17H SLOPES OF R.M.A./)
    KL=2
560 DO 570 J=1,JK
570 R(J)=RMA(J)
    GO TO 490
580 PAUSE
    IF(SENSE SWITCH 4)70,10
    END
```

APPENDIX IV.

Stepwise regression analysis programme.

A. Description of the programme.

The statistical method used in this thesis for both stepwise regression analyses (SECTION IV-F) and trend analysis (SECTION IV-E) was adapted from the procedure given by Miesch and Connor (1968) which was, in turn, based on the method proposed by Efroymson (1960).

The programme was written as five subprogrammes (APPENDIX IV B) firstly because of limited computer storage and secondly to increase the versatility of the programme. The main loop of the programme (Main 3) could not be further reduced in size and the relatively small storage of the computer (40K) limited the programme to a total of 35 variables. Conversion between trend analysis and regression analysis applications was achieved by making alterations to the "read" block of Main 1 and to the "printout" programme, Main 5. No changes to the other programmes were necessary.

The essential functions of each programme are summarised in APPENDIX IV B and a listing of the programmes is given in Appendix IV C.

The insertion of a particular independent variable into the regression equation depends on that variable making a significant improvement to the equation, and the level of significance, QF, of this improvement is derived from the variance ratio, F, by the subroutine PROB. The approximations used in PROB are valid only for more than 100 samples and this is a limitation of the programme although trend analysis or regression analysis with less than 100 sites was not undertaken in this thesis.

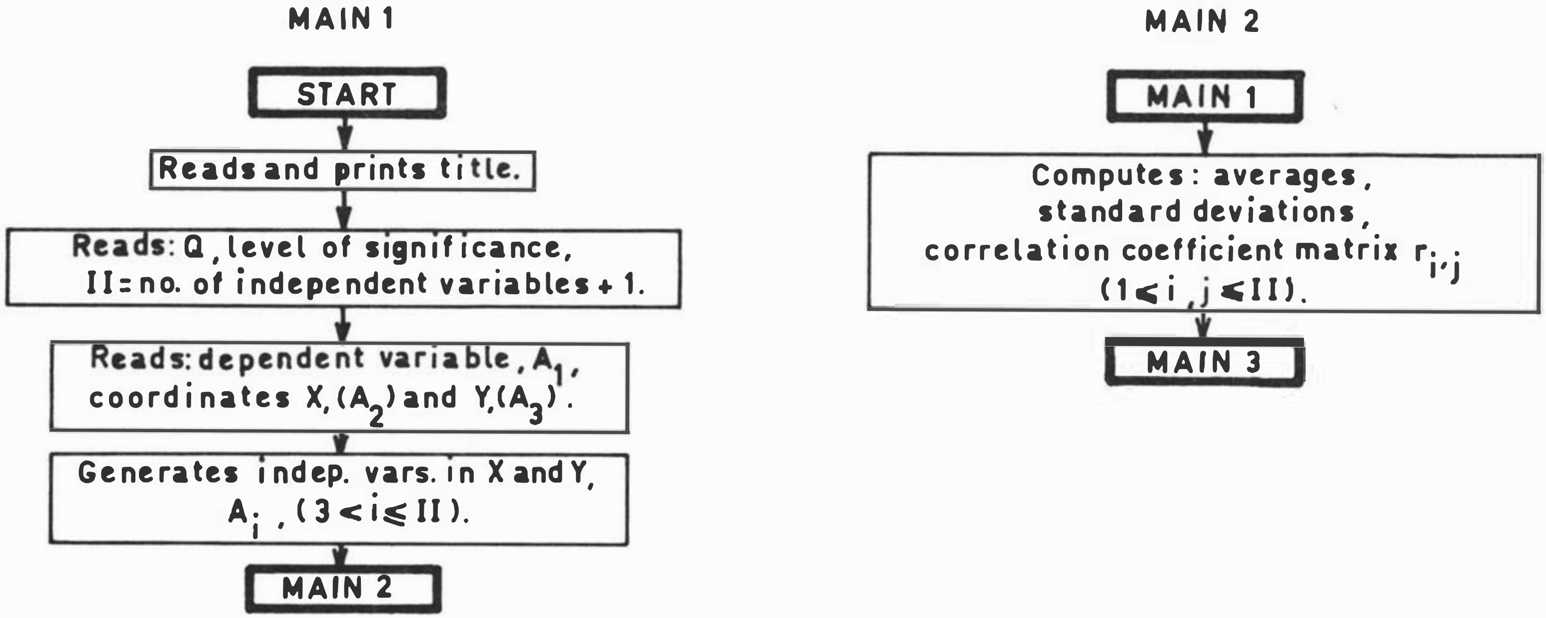
Miesch and Connor (1968) pointed out that serious errors in the regression coefficients can arise during matrix computations, but that if regularly distributed sampling sites are used and sufficient significant figures are carried in the computations, then these errors can be reduced. The sites used in the present study were regularly spaced and 11 significant figures were carried in the calculations.

For trend analysis, the same data decks as described for the correlation coefficient programme, were used except that for each site the coordinates of that site were punched onto a card which was placed immediately behind the data card corresponding to the site. The east-west coordinates shown in Figure III - 1 were increased by one to eliminate the zero coordinate. The particular metal concentration to be read off the data cards was specified on a control card prior to the data. The independent terms in the coordinates X and Y which were tested for inclusion in the regression equations were as follows:

linear	X, Y
quadratic	X^2, XY, Y^2
cubic	X^3, X^2Y, XY^2, Y^3
quadratic	X^4, X^3Y, X^2Y^2, XY^3, Y^4
quintic	X^5, X^4Y, X^3Y^2, X^2Y^3, XY^4, Y^5
square root	$X^{\frac{1}{2}}$ $(XY)^{\frac{1}{2}}$ $Y^{\frac{1}{2}}$
reciprocal	X^{-2}, X^{-1}, $(XY)^{-1}$, Y^{-1}, Y^{-2}
logarithmic	$(\log X)^2$, $\log X$, $\log X.\log Y$, $\log Y$, $(\log Y)^2$

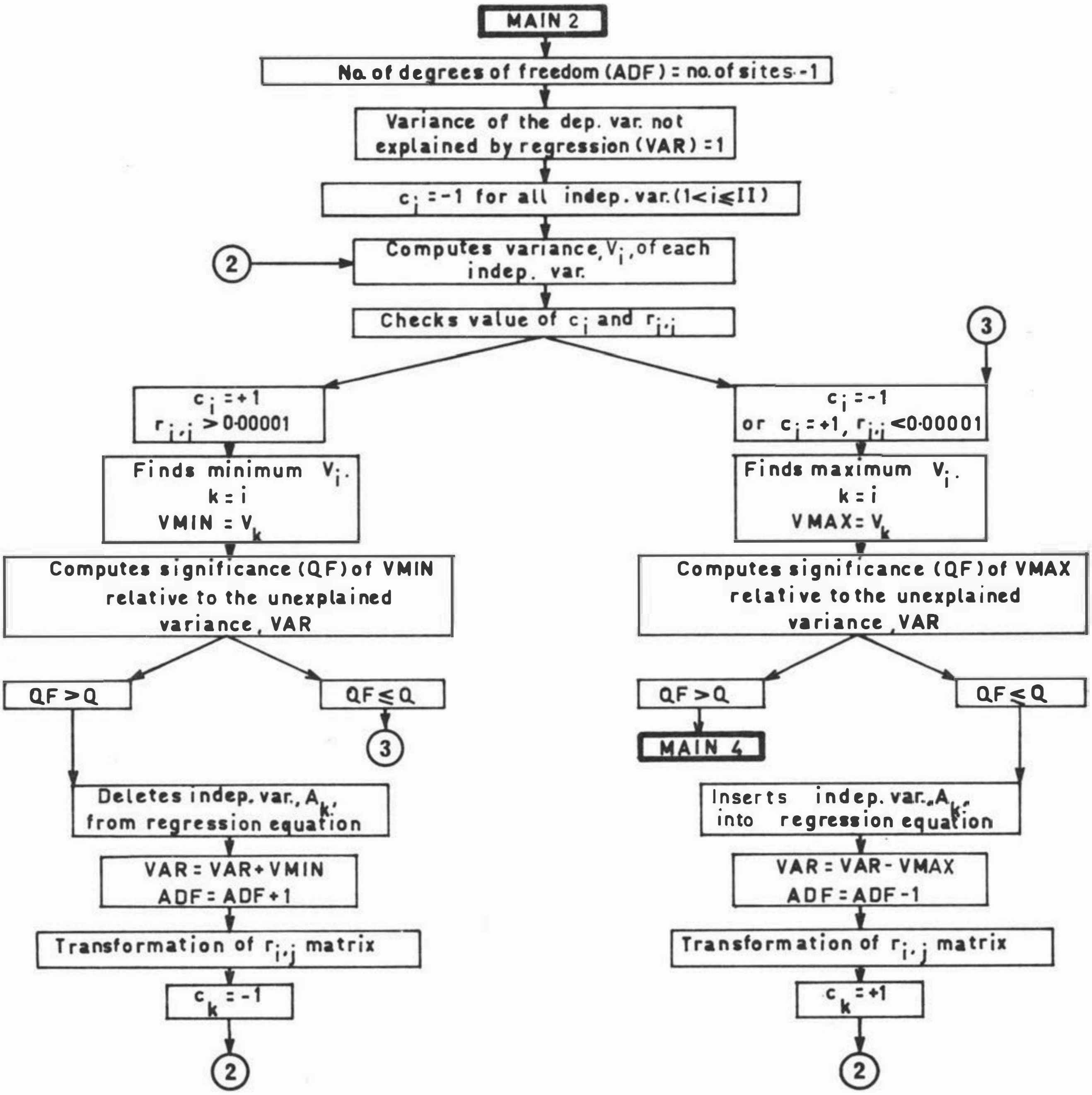
For regression analysis, the required variables for each site were read successively off the corresponding plant data card, soil data card and the card containing the physical variables.

The control cards to precede the data deck are explained at the beginning of the programme listing (APPENDIX IV C). Although only disk storage limits the number of sample sites used, the programme requires a running time of two hours for a data deck containing 150 samples if 35 variables are used.

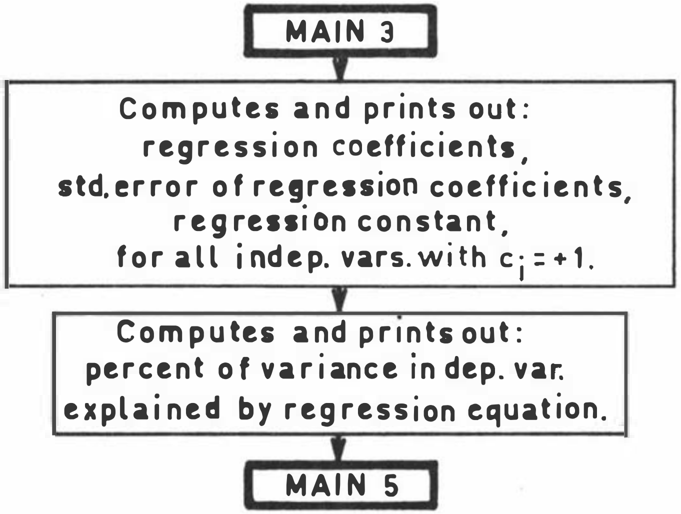


Appendix IVB. Summary of the essential procedures involved in each main programme.

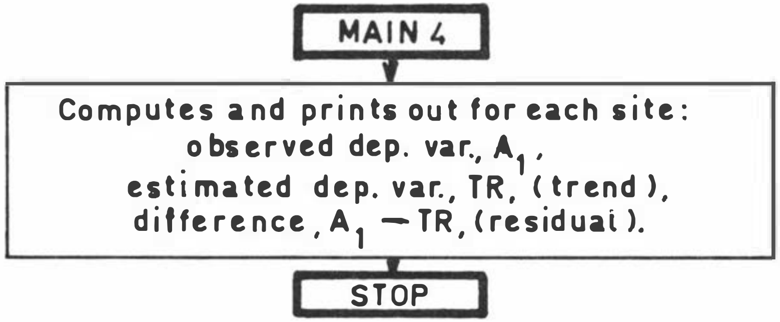
MAIN 3


MAIN 2
No. of degrees of freedom (ADF) = no. of sites -1
Variance of the dep. var. not explained by regression (VAR) = 1
$c_i = -1$ for all indep. var. $(1 < i \leq II)$
2
Computes variance, V_i, of each indep. var.
Checks value of c_i and $r_{i,i}$
3
$c_i = +1$ $r_{i,i} > 0{\cdot}00001$
$c_i = -1$ or $c_i = +1$, $r_{i,i} < 0{\cdot}00001$
Finds minimum V_i. $k = i$ VMIN = V_k
Finds maximum V_i. $k = i$ VMAX = V_k
Computes significance (QF) of VMIN relative to the unexplained variance, VAR
Computes significance (QF) of VMAX relative to the unexplained variance, VAR
QF > Q
QF ≤ Q
3
QF > Q
QF ≤ Q
MAIN 4
Deletes indep. var., A_k, from regression equation
Inserts indep. var., A_k, into regression equation
VAR = VAR + VMIN ADF = ADF + 1
VAR = VAR - VMAX ADF = ADF - 1
Transformation of $r_{i,j}$ matrix
Transformation of $r_{i,j}$ matrix
$c_k = -1$
$c_k = +1$
2
2

MAIN 4


MAIN 3
Computes and prints out:
regression coefficients,
std. error of regression coefficients,
regression constant,
for all indep. vars. with $c_i = +1$.
Computes and prints out:
percent of variance in dep. var.
explained by regression equation.
MAIN 5

MAIN 5


MAIN 4
Computes and prints out for each site:
observed dep. var., A_1,
estimated dep. var., TR, (trend),
difference, $A_1 - TR$, (residual).
STOP

APPENDIX IVC

STEPWISE MULTIPLE REGRESSION PROGRAMME USED FOR TREND ANALYSIS AND MULTIPLE REGRESSION ANALYSIS

FORTRAN IID

```
C     MAIN 1
C
C     TREND SURFACE ANALYSIS USING  STEPWISE MULTIPLE REGRESSION
C     A(1) IS DEPENDENT VARIABLE , A(2) TO A(35) ARE INDEPENDENT
C     VARIABLES
C     CARDS TO PRECEDE DATA DECK ARE AS FOLLOWS
C     TITLE CARD   TITLE MAY OCCUPY UP TO 79 COLUMNS
C     CARD CONTAINING VARIABLES II AND Q IN FORMAT (I6,F6.0)
C     II= TOTAL NUMBER OF DEPENDANT PLUS INDEPENDANT VARIABLES TO BE
C     READ IN AND GENERATED
C     Q= PROBABILITY LEVEL AT WHICH VARIABLES ARE TO BE ADDED OR DELETED
C     FROM THE REGRESSION EQUATION
C     CARD CONTAINING IFOR
C     IFOR= READ FORMAT FOR THE DEPENDANT VARIABLE
C     THE X AND Y COORDINATES ARE PUNCHED IN FORMAT(2F6.0) ONTO A CARD
C     IMMEDIATELY FOLLOWING THE CARD CONTAINING THE DEPENDANT VARIABLE
C     FOR THOSE COORDINATES
C     TWO CARDS CONTAINING A NUMERAL 9 IN COLUMN ONE MUST FOLLOW DATA
      DIMENSION A(35),IFOR(20)
      COMMON A,II,ISAVE,Q
      DEFINE DISK(15,1000)
C
C     READS TITLE AND CONTROL CARDS
C
 1000 READ 20
   20 FORMAT (80H
     1                           )
      PRINT 20
      READ 23,II,Q
   23 FORMAT(1X,I5,F6.0)
      PRINT 26,Q
   26 FORMAT (19H PROBABILITY LEVEL=,F6.2)
      READ 25,IFOR
   25 FORMAT(20A1)
C
C     INITIALISES VARIABLES
C
   11 DO 61 I=1,35
   61 A(I)=0.0
C
C     READS DEP. VAR. AND X,Y COORDINATES
```

```
C       GENERATES INDEP. VARS.
C
      IDEX=1
   24 READ IFOR,M,A(1)
      READ 22,M,A(2),A(3)
   22 FORMAT (I1,F5.0,F6.0)
      IF(M-9)50,60,60
   50 A(1)=0.43429448*LOGF(A(1))
      A(4)=A(2)**2
      A(5)=A(2)*A(3)
      A(6)=A(3)**2
      A(7)=A(2)**3
      A(8)=A(3)*A(2)**2
      A(9)=A(2)*A(3)**2
      A(10)=A(3)**3
      A(11)=A(2)**4
      A(12)=A(2)**3*A(3)
      A(13)=A(2)**2*A(3)**2
      A(14)=A(2)*A(3)**3
      A(15)=A(3)**4
      A(16)=A(2)**5
      A(17)=A(2)**4*A(3)
      A(18)=A(2)**3*A(3)**2
      A(19)=A(2)**2*A(3)**3
      A(20)=A(2)*A(3)**4
      A(21)=A(3)**5
      A(22)=SQRTF(A(2))
      A(23)=SQRTF(A(3))
      A(24)=SQRTF(A(2)*A(3))
      A(25)=0.43429448*LOGF(A(2))
      A(26)=0.43429448*LOGF(A(3))
      A(27)=0.43429448**2*LOGF(A(2))*LOGF(A(3))
      A(28)=0.43429448**2*(LOGF(A(2))**2)
      A(29)=0.43429448**2*(LOGF(A(3))**2)
      A(30)=1.0/A(2)
      A(31)=1.0/A(3)
      A(32)=1.0/(A(2)**2)
      A(33)=1.0/(A(3)**2)
      A(34)=1.0/(A(2)*A(3))
      RECORD(IDEX)A
      GO TO 24
   60 ISAVE=IDEX
      CALL LINK(MAIN 2)
      END
```

```
C     MAIN 2
C
      DIMENSION A(35),AV(35),SQ(35),R(35,35),SD(35)
      COMMON A,II,ISAVE,Q,AV,R,AN,SQ,SD
      DEFINE DISK(15,1000)
C
C     COMPUTES  MEANS,STANDARD DEVIATIONS,CORRELATION COEFFICIENTS
C
      DO 60 I=1,II
      SQ(I)=0.0
      SD(I)=0
   60 AV(I)=0
      DO 62 I=1,II
      DO 62 J=1,II
   62 R(I,J)=0.0
      AN=0.0
      IDEX=1
  100 IF(IDEX-ISAVE)101,104,104
  101 FETCH(IDEX)A
      AN=AN+1.0
      DO 102 I=1,II
      AV(I)=AV(I)+A(I)
  102 SQ(I)=SQ(I)+A(I)**2
      DO 103 I=1,II
      DO 103 J=1,II
  103 R(I,J)=R(I,J)+A(I)*A(J)
      GO TO 100
  104 DO 105 I=1,II
      AV(I)=AV(I)/AN
  105 SD(I)=SQRTF((SQ(I)-AN*AV(I)**2)/(AN-1.0))
      DO 107 I=1,II
      DO 107 J=1,II
  107 R(I,J)=(R(I,J)-AN*AV(I)*AV(J))/SQRTF((SQ(I)-AN*AV(I)**2)*(SQ(J)-
     1AN*AV(J)**2))
      I=1
      PRINT 110,(R(I,J),J=1,II)
  110 FORMAT(12E10.0/)
      CALL LINK(MAIN 3)
      END


C     MAIN 3
C
      DIMENSION A(35),AV(35),SQ(35),SD(35),R(35,35),V(35),C(35)
      COMMON A,II,ISAVE,Q,AV,R,AN,SQ,SD,C
      DEFINE DISK(15,1000)
      ADF=AN-1.0
      VAR=1.0
```

```
      PRINT 92
   92 FORMAT (20X,8HVARIABLE,12X,2HQF/)
      DO 200 I=2,II
  200 C(I)=-1.0
  203 SQ(1)=SD(1)*SQRTF(R(1,1)/ADF)
      DO 202 I=2,II
  202 V(I)=(R(I,1)**2)/R(I,I)
      VMIN=.9E99
      I=2
  500 IF(C(I)-1.0)501,503,501
  501 I=I+1
      IF(I-II)502,502,506
  502 GO TO 500
  503 IF(R(I,I)-.1E-4)501,501,504
  504 IF(V(I)-VMIN)505,501,501
  505 VMIN=V(I)
      K=I
      GO TO 501
  506 IF(VMIN-.9E99)508,399,399
  508 F=(VMIN*ADF)/VAR
      CALL PROB(F,ADF,QF)
      IF(QF-Q)399,507,507
  507 VAR=VAR+VMIN
      ADF=ADF+1.0
      PRINT 90,K,QF
   90 FORMAT (8H DELETED,16X,I3,10X,E14.0)
      GO TO 601
  399 VMAX=.9E-99
      I=2
  400 IF(C(I)+1.0)401,403,401
  401 I=I+1
      IF(I-II)402,402,406
  402 GO TO 400
  403 IF(R(I,I)-.1E-4)401,401,404
  404 IF(V(I)-VMAX)401,401,405
  405 VMAX=V(I)
      K=I
      GO TO 401
  406 IF(VMAX-.9E-99)900,701,95
   95 F=(VMAX*ADF)/(VAR-VMAX)
      CALL PROB(F,ADF,QF)
      IF(QF-Q)407,407,701
  407 VAR=VAR-VMAX
      ADF=ADF-1.0
      PRINT 91,K,QF
   91 FORMAT (6H ADDED,18X,I3,10X,E14.0)
  601 IK=K+1
      IJ=K-1
      IF(IK-II)98,98,99
```

```
   98 DO 607 I=IK,II
      DO 607 J=I,II
  607 R(I,J)=R(I,J)-((C(K)*C(I)*R(K,J)*R(K,I))/R(K,K))
      DO 700 I=IK,II
  700 R(I,1)=R(I,1)-((C(K)*C(I)*R(K,1)*R(K,I))/R(K,K))
      IF(IJ-2)96,97,97
   97 DO 604 I=2,IJ
      DO 604 J=IK,II
  604 R(I,J)=R(I,J)-((R(K,J)*R(I,K))/R(K,K))
   99 DO 602 I=2,IJ
      DO 602 J=I,IJ
  602 R(I,J)=R(I,J)-((C(K)*C(J)*R(I,K)*R(J,K))/R (K,K))
      DO 608 I=2,IJ
  608 R(I,1)=R(I,1)-((R(K,1)*R(I,K))/R(K,K))
      DO 603 I=2,IJ
  603 R(I,K)=-R(I,K)/R(K,K)
   96 R(K,1)=R(K,1)/R(K,K)
      IF(IK-II)94,94,93
   94 DO 606 J=IK,II
  606 R(K,J)=R(K,J)/R(K,K)
   93 R(K,K)=1.0/R(K,K)
      C(K)=-C(K)
      GO TO 203
  701 CALL LINK(MAIN 4)
  900 TYPE 302
  302 FORMAT(20H VMAX LESS THAN ZERO/)
      END


      SUBROUTINE PROB(F,ADF,QF)
      IF(F-.9E-99)10,10,30
   10 QF=0.0
      GO TO 20
   30 AM=1.0
    1 FAC=F**0.33333333333
      A=0.2222222222/AM
      B=0.2222222222/ADF
      W=(FAC*(1.0-B)-(1.0-A))/SQRTF(A+B*FAC**2)
      T=1.0/(1.0+0.2316419*ABSF(W))
      ZX=0.3989422804*EXPF(-0.5*W*W)
      FAC=((((1.330274429*T-1.821255978)*T+1.781477937)*T-0.356563782)*T
     1+0.319381530)*T
      FAC=FAC*ZX
      IF(W)2,3,3
    2 QF=1.0-FAC
    3 QF=FAC
   20 RETURN
      END
```

```
C     MAIN 4
C
      DIMENSION A(35),AV(35),SQ(35),SD(35),R(35,35),B(35),C(35)
      COMMON A,II,ISAVE,Q,AV,R,AN,SQ,SD,C,B
      DEFINE DISK(15,1000)
C
C     COMPUTES REGRESSION CONSTANT AND COEFFICIENTS
C     COMPUTES ERROR OF REGRESSION CONSTANT
C     COMPUTES PERCENT SUM OF SQUARES EXPLAINED
C
      I=2
      DX=0.0
      PSS=0.0
      PRINT 205
  205 FORMAT (9HOVARIABLE,5X,22HREGRESSION COEFFICIENT,4X,31HSTD. ERROR
     1OF REGRESSION COEFF./)
  702 IF(C(I)-1.0)703,704,703
  703 IF(I-II)706,705,705
  706 I=I+1
      GO TO 702
  704 L=I
      B(L)=(R(L,1)*SD(1))/SD(L)
      SQ(L)=(SQ(1)*SQRTF(R(L,L)))/SD(L)
      PRINT 208,L,B(L),SQ(L)
  208 FORMAT(I5,13X,E14.0,18X,E14.0)
      DX=DX+(B(L)*AV(L))
      PSS=PSS+(R(L,1)*R(1,L))
      GO TO 703
  705 B(1)=AV(1)-DX
      PSS=100.0*PSS
      PRINT 206,B(1)
  206 FORMAT (21HOREGRESSION CONSTANT=,E14.0/)
      PRINT 207,PSS
  207 FORMAT (34H PERCENT SUM OF SQUARES EXPLAINED=,F10.4//)
      CALL LINK(MAIN 6)
      END
```

```
C     MAIN 5
C
      DIMENSION A(35),AV(35),SQ(35),SD(35),R(35,35),B(35),C(35)
      COMMON A,II,ISAVE,Q,AV,R,AN,SQ,SD,C,B
      DEFINE DISK(15,1000)
C
C     COMPARES OBSERVED AND COMPUTED VALUES OF DEPENDANT VARIABLE
C
      PRINT 209
  209 FORMAT (9H OBSERVED,3X,1HX,5X,1HY,2X,8HCOMPUTED,2X,8HRESIDUAL/)
      IDEX=1
  800 TR=B(1)
      IF(IDEX-ISAVE)801,900,900
  801 FETCH(IDEX)A
      I=2
  807 IF(C(I)-1.0)803,804,803
  803 IF(I-II)806,805,805
  806 I=I+1
      GO TO 807
  804 TR=TR+(B(I)*A(I))
      GO TO 803
  805 A(1)=EXPF(A(1)/0.43429448)
      TR=EXPF(TR/0.43429448)
      RESID=A(1)-TR
      PRINT 210,A(1),A(2),A(3),TR,RESID
  210 FORMAT (F8.2,I5,I6,F9.2,F10.2)
      GO TO 800
  900 PAUSE
      END
```

APPENDIX V.

Programme to compute the probability of site overlap.

A. Description of the programme.

The method used in SECTION IV - G to evaluate the ratios of the metal concentrations in the plants as indicators of high copper concentrations in the soil, depended on the degree of overlap between the sampling sites corresponding to the anomalous plant values and the anomalous soil values.

The essential logic involved in this technique was as follows (letters in parenthesis correspond to those used in the programme). A set of 147 sampling sites (S) contained 44 sites (R) at which anomalous concentrations of copper occurred. Fortyfour sites (T) corresponding to the anomalous plant values, were selected out of the set (S) of soil sites. What was the chance that some (P) of the sites selected would be anomalous soil sites? If a relationship existed between the T sites selected from the set of soil sites (S) and the R anomalous soil sites, then the number of anomalous soil sites found in the T sites selected would be greater than the number found if no relationship existed. The probability of finding P anomalous soil sites in the T sites selected is given by [*]

$$\text{PROB} = \frac{R!}{P!\,(R-P)!} \cdot \frac{(S-R)!}{(T-P)!\,(S-R-T+P)!} \cdot \frac{T!\,(S-T)!}{S!}$$

Values of R, S, T and P are read in with the programme listed in APPENDIX V B and this will compute PROB for values of P from the value read in up to P=T (or until very small numbers are encountered).

[*] The theory behind the derivation of this equation is given in Adler,I.,1969,Probability and statistics for everyman, London, Dennis Dobson.

APPENDIX VB

```
C     PROGRAMME TO COMPUTE THE PROBABILITY OF OBTAINING P SAMPLES
C     OF TYPE L IN A RANDOM SUBSET OF T SAMPLES, TAKEN FROM A POPULATION
C     CONSISTING OF R SAMPLES OF TYPE L AND M SAMPLES OF TYPE U.
C     S=R+M
C
C     INPUT DATA CONSISTS OF R,S,T,P ON ONE CARD IN FORMAT (4I5)
C
C
      P=0.0
      R=0.0
      S=0.0
      T=0.0
      READ 1,R,S,T,P
      AB=R
      AC=S
      AD=T
      AE=P
  100 A=R
      R=0.43429448*LOGF(R)
    3 A=A-1.0
      R=R+0.43429448*LOGF(A)
      IF(A-AB+AE-1.0)4,4,3
    4 B=S
      S=0.43429448*LOGF(S)
    5 B=B-1.0
      S=S+0.43429448*LOGF(B)
      IF(B-AC+AD-AE-1.0)7,7,5
    7 C=AD
      T=0.43429448*LOGF(T)
    8 C=C-1.0
      T=T+0.43429448*LOGF(C)
      IF(C-2.0)10,10,8
   10 D=AE
      P=0.43429448*LOGF(P)
   11 D=D-1.0
      P=P+0.43429448*LOGF(D)
      IF(D-2.0)13,13,11
   13 Q=AD-AE
      E=AD-AE
      Q=0.43429448*LOGF(Q)
   14 E=E-1.0
      Q=Q+0.43429448*LOGF(E)
      IF(E-2.0)16,16,14
   16 U=AB+AC
      F=U
      U=0.43429448*LOGF(U)
```

```
   17 F=F-1.0
      U=U+0.43429448*LOGF(F)
      IF(F-AB-AC+AD-1.0)19,19,17
   19 PROB=R+S+T-P-Q-U
      PROB=EXPF(PROB/0.43429448)
      PRINT 20,AE,PROB
      IF(AE-AD)21,22,22
   21 R=AB
      S=AC
      T=AD
      AE=AE+1.0
      P=AE
      GO TO 100
    1 FORMAT(4I5)
   20 FORMAT(I5,5X,E10.0)
   22 CALL EXIT
      END
```

APPENDIX VI
DATA LISTING

NOTHOFAGUS LEAVES

IDENTIFICATION	N.TRUNCATA	N.FUSCA	N.MENZIESII
MARCH 1969	11	21	31
NOVEMBER 1969	12	22	32
NOVEMBER 1970	13	23	33

N.TRUNCATA

SITE NO.	IDENT.	NI	CO	CU	ZN	CR	FE	CA	MG	MN	K	ASH
		PPM	PPM	PPM	PPM	PPM	PPM	PCENT.	PCENT.	PCENT.	PCENT.	PCENT.
18	12	50	13	55	240	17	1100	18.5	4.50	1.10	6.9	5.54
19	12	55	15	85	320	10	1300	20.5	5.50	1.25	10.5	4.69
110	12	70	14	75	390	10	950	22.0	4.50	1.25	8.2	4.40
111	12	50	12	73	260	10	1350	20.5	3.50	2.25	8.4	4.56
111	11	100	20	75	315	7	1650	21.5	3.50	1.10	3.3	5.60
112	12	105	10	145	270	12	1700	16.5	4.00	1.25	9.2	5.00
113	12	140	13	115	250	14	1700	18.5	3.00	1.25	10.3	6.29
114	12	120	13	80	260	9	950	20.0	3.25	1.10	7.9	7.63
115	12	365	14	120	275	16	1150	23.0	4.00	1.75	10.0	6.16
116	12	95	13	63	235	13	1250	25.5	4.00	1.90	8.4	6.57
117	12	90	6	155	220	9	1200	11.5	2.25	.65	8.7	5.33
118	13	270	18	80	350	15	1050	16.5	7.25	.70	8.6	4.61
120	13	155	18	90	315	10	1000	20.0	2.25	.75	11.2	6.21
29	12	120	13	70	250	15	1450	21.0	6.00	1.00	7.4	4.35
210	12	110	14	68	300	8	1050	25.5	5.75	.50	9.2	4.65
210	11	220	21	53	120	8	734	20.0	3.96	1.06	8.6	5.28
211	12	80	14	45	150	7	550	29.5	4.50	1.40	7.4	6.86
211	11	115	19	80	200	7	773	23.5	4.00	.89	4.4	5.00
212	12	215	16	85	280	10	1650	25.0	4.00	1.50	11.3	5.26
212	11	273	19	85	250	8	1505	20.0	3.75	.82	5.5	4.77
213	12	55	13	60	260	9	1000	23.5	4.00	1.50	7.4	5.11
213	11	138	15	75	200	10	890	19.5	3.35	1.23	4.6	4.32
214	12	220	15	80	270	11	1500	21.0	5.75	1.50	10.0	4.26
215	12	130	13	63	290	10	1500	20.5	5.57	1.40	7.6	4.26
312	11	128	25	85	250	10	1275	26.5	3.75	1.23	4.6	4.65
314	12	170	12	65	250	11	1000	23.0	4.25	1.15	7.4	5.87
315	12	185	10	95	280	11	1150	15.5	5.75	1.30	8.7	3.32
316	12	110	14	75	270	9	950	26.0	4.75	1.50	9.5	6.20
48	12	70	12	65	280	7	800	23.0	5.00	1.30	7.1	4.80
48	11	110	19	100	275	7	890	23.0	4.50	1.03	6.5	4.34
49	12	115	14	70	260	8	850	26.5	4.00	1.75	9.0	5.68
49	11	120	17	90	200	4	790	21.5	3.50	1.23	4.4	5.78

410	12	50	11	68	210	8	950	20.5	4.25	1.75	10.0	4.67
411	12	70	15	70	200	8	900	26.0	3.85	1.25	10.3	5.77
414	12	210	17	90	320	14	750	26.5	4.00	2.00	16.6	6.21
415	12	80	12	63	140	10	700	25.5	3.50	1.25	9.8	6.24
416	12	125	13	65	240	8	900	25.0	3.75	1.10	8.2	6.37
417	13	115	18	75	240	10	700	21.5	4.75	.85	7.7	9.02
419	13	160	20	85	275	23	1300	19.0	4.00	.70	10.1	4.66
56	12	85	12	85	290	8	1100	20.0	4.25	1.60	12.4	3.88
515	11	157	13	115	225	5	1130	13.0	7.50	.64	8.9	5.14
516	12	210	13	128	250	8	750	25.0	4.25	1.00	9.8	5.02
516	11	228	14	170	235	10	925	21.5	4.35	.75	6.5	4.97
518	13	75	20	60	180	13	700	24.5	3.50	.70	8.0	5.83
519	13	110	20	70	215	10	850	23.0	3.75	.65	8.0	5.32
520	13	75	18	75	300	15	800	19.5	5.25	.60	11.2	4.78
616	12	260	17	700	550	19	2700	25.0	5.25	1.00	13.5	4.95
79	13	40	15	65	190	10	900	21.0	2.25	1.60	8.3	5.88
710	13	75	20	55	245	10	650	27.0	3.25	1.35	8.0	6.19
711	13	60	13	60	240	10	1000	15.5	4.75	1.40	7.5	5.47
712	13	120	15	80	170	10	1000	16.0	2.50	1.35	11.5	6.42
714	13	140	15	65	230	10	950	16.5	3.50	1.10	9.2	5.54
715	13	195	20	75	275	15	850	18.5	3.25	1.00	9.2	7.10
716	13	80	23	60	140	10	950	22.0	3.00	.80	8.3	6.43
717	13	90	20	60	220	15	750	22.0	3.00	1.00	8.0	4.87
718	13	60	18	50	180	15	1250	18.0	3.50	.70	9.2	6.22

N.FUSCA

SITE NO.	IDENT.	NI PPM	CO PPM	CU PPM	ZN PPM	CR PPM	FE PPM	CA PCENT.	MG PCENT.	MN PCENT.	K PCENT.	ASH PCENT.
11	22	60	14	95	300	14	1200	19.5	6.00	1.25	11.9	5.25
13	21	320	14	170	330	6	1505	22.5	6.00	.42	6.5	3.84
14	22	100	15	155	550	15	1100	22.5	8.00	2.00	11.6	4.30
15	22	130	15	135	370	19	1800	22.0	6.75	2.00	11.9	4.29
15	21	100	18	135	390	8	1180	24.5	5.75	2.67	5.5	5.15
16	22	45	10	1400	270	13	1650	19.0	6.25	2.25	9.0	5.01
17	22	85	9	100	310	6	850	14.5	5.00	1.00	9.5	5.06
17	21	161	24	120	280	7	1003	24.5	4.25	1.64	4.6	4.56
110	21	138	20	100	275	6	890	5.0	4.85	1.85	4.4	4.67
112	21	120	19	85	250	15	2000	18.5	3.25	1.10	4.6	5.40
113	21	157	20	100	180	9	1450	19.5	3.15	.89	4.4	5.66
20	23	60	18	100	620	15	1650	21.0	4.50	1.50	11.2	5.61
21	22	120	13	95	440	13	1900	18.5	5.25	2.10	12.4	4.99
21	21	90	20	100	390	9	1735	22.5	5.00	1.44	5.9	4.40
22	22	65	11	90	440	10	900	20.0	5.00	1.85	10.8	4.69
22	21	75	18	90	225	4	925	21.5	3.50	1.30	4.0	6.05
23	22	60	12	93	275	11	1000	19.5	5.50	1.85	12.6	4.26
24	22	140	16	120	450	17	2050	22.5	6.25	2.00	10.5	4.35
28	21	560	23	660	390	15	4000	13.5	5.75	.50	10.0	4.66
29	21	110	22	85	210	12	1275	19.0	3.25	.75	6.5	4.76
216	22	115	17	100	650	21	1650	27.5	5.50	2.40	9.2	5.57
216	21	120	21	90	235	7	1450	20.5	3.15	1.30	6.3	7.37
217	22	220	14	105	315	13	1600	20.0	5.75	.75	11.9	4.86
218	23	205	13	100	260	20	1000	13.5	3.00	.75	10.6	7.66
219	23	80	13	95	275	15	1250	14.5	6.50	1.00	11.5	4.71
220	23	105	15	90	265	15	1250	17.5	6.50	.70	10.2	5.04
30	23	45	18	95	300	20	1550	22.0	4.75	2.40	10.8	4.99
34	22	240	11	123	300	8	750	14.5	4.25	1.80	14.5	4.56
38	22	35	11	68	250	8	600	17.5	4.50	1.75	11.1	6.80
38	21	75	18	110	250	10	825	20.0	4.80	1.78	6.9	4.91
311	22	255	10	95	330	8	900	13.5	6.75	.50	11.3	4.48
311	21	185	16	85	315	7	760	18.5	5.75	1.37	5.0	5.55
312	22	135	6	63	160	8	500	7.5	2.75	.80	10.5	4.50
314	21	157	21	85	275	13	890	25.0	4.15	2.74	5.0	4.70
316	21	110	18	60	190	5	825	22.0	3.00	.75	3.6	5.86
317	23	120	20	90	330	25	2300	22.5	5.00	1.40	10.8	5.59
318	23	95	20	80	310	15	1100	20.5	5.25	.95	10.2	5.71
319	23	625	18	130	260	23	1850	13.0	10.00	.30	10.9	3.59
320	23	105	15	75	285	15	1050	16.0	5.50	.70	8.0	5.72
40	23	45	15	80	330	10	1450	17.0	5.00	2.30	8.9	5.34
41	21	78	22	115	440	13	2650	22.0	4.10	2.54	6.9	5.11
41	22	45	13	90	390	10	1450	21.0	5.00	3.00	14.2	4.13

43	22	80	13	110	250	14	1400	22.5	5.00	4.00	11.6	3.98
45	21	90	22	130	360	11	2000	19.5	5.75	1.71	8.1	4.50
46	22	35	12	78	260	9	1200	20.0	5.00	1.75	9.2	5.65
47	22	55	12	88	390	9	1000	23.0	6.00	2.40	11.9	3.93
410	21	57	18	75	200	5	760	19.0	3.15	1.44	4.6	5.88
413	22	105	12	63	220	10	650	23.5	5.50	1.50	9.5	6.11
413	21	295	14	135	300	8	890	21.5	8.75	.60	10.5	4.14
414	21	185	21	80	235	11	890	21.5	3.50	1.10	5.9	5.04
416	21	81	18	60	150	5	760	22.5	3.00	.68	3.6	6.68
418	23	80	20	90	320	15	1100	19.0	6.00	1.35	9.2	5.48
50	23	30	18	85	340	10	700	21.0	5.25	3.70	10.8	4.91
51	22	70	13	88	275	11	1850	23.5	4.50	2.00	10.0	4.93
52	22	80	13	95	270	10	1150	20.0	4.50	1.50	12.1	4.61
56	21	240	25	130	330	10	1650	17.0	5.50	1.71	7.4	4.77
58	22	135	13	90	220	8	600	16.0	4.00	1.40	18.7	7.18
58	21	128	20	110	190	8	1000	18.0	4.65	1.99	9.4	6.67
59	22	90	10	75	250	8	1200	17.5	4.25	1.25	11.6	5.35
510	22	60	14	80	300	8	1300	24.5	5.25	1.00	10.5	5.11
514	22	255	10	250	300	10	1250	19.5	5.75	.40	11.1	4.85
575	21	295	19	143	250	5	1050	19.0	4.10	1.30	8.1	4.93
60	23	40	18	75	385	10	700	21.0	4.50	1.90	8.0	5.70
61	22	25	12	63	260	10	1350	24.5	4.50	1.00	9.2	4.86
64	22	45	13	75	280	8	960	26.0	5.25	1.25	11.9	4.19
65	22	50	13	73	420	9	650	26.5	4.75	.75	10.5	6.01
65	21	120	20	143	340	9	1575	22.0	5.10	1.51	6.3	4.25
66	22	95	12	68	265	10	950	25.5	6.00	1.50	8.5	5.80
68	22	55	13	95	390	9	1200	23.5	5.50	1.75	12.6	3.63
68	21	65	19	110	300	7	1230	20.5	4.10	1.58	6.5	4.24
69	21	75	18	60	150	9	600	24.0	9.75	.62	3.6	4.13
611	22	90	10	70	250	8	800	19.5	5.00	2.00	10.8	5.30
612	22	80	12	68	280	10	1050	21.0	4.75	1.25	10.8	5.71
613	22	80	12	128	400	9	1200	22.5	5.57	1.25	11.6	4.00
613	21	110	16	90	250	7	1230	20.0	5.40	1.58	5.5	4.80
614	22	240	10	100	210	11	800	15.5	6.75	1.25	13.2	5.66
614	21	200	19	143	235	7	1505	17.5	5.90	1.23	7.4	4.20
615	21	100	19	90	140	29	3275	12.5	4.00	.68	4.4	7.14
617	21	305	13	110	200	6	1130	14.0	6.00	.41	5.9	5.13
618	21	450	14	115	200	6	825	16.0	6.60	.41	7.4	4.50
619	21	892	12	153	240	7	1400	16.0	4.75	.60	11.5	3.85
620	23	140	18	110	460	20	2100	18.0	4.00	.60	10.8	4.60
70	23	140	18	105	380	15	1300	19.5	5.75	2.20	10.8	4.50
71	23	70	18	95	320	15	1200	19.5	5.50	1.70	9.2	5.65
72	23	55	20	90	430	18	950	25.5	3.50	1.90	9.2	5.04
74	23	95	18	70	225	13	600	19.0	4.25	1.00	8.3	5.70
713	23	65	15	65	310	13	700	19.5	4.00	1.35	8.3	6.45
719	23	200	13	80	350	15	1000	19.5	5.25	.50	7.5	5.33

N.MENZIESII

SITE NO.	IDENT.	NI PPM	CO PPM	CU PPM	ZN PPM	CR PPM	FE PPM	CA PCENT.	MG PCENT.	MN PCENT.	K PCENT.	ASH PCENT.
10	33	185	20	125	600	15	1800	19.5	5.25	1.30	14.3	3.03
11	31	343	30	210	820	16	2650	20.0	3.75	.76	5.9	3.02
13	32	295	23	150	740	18	2500	22.5	6.50	1.25	10.0	3.22
14	31	285	23	225	765	7	2340	21.0	4.25	1.44	10.0	3.28
18	31	175	19	143	650	12	1800	19.0	3.50	1.85	9.6	3.07
19	31	185	23	115	625	16	1900	5.0	3.70	1.78	6.5	3.49
114	31	320	24	210	545	26	2825	19.0	6.75	1.64	7.8	2.79
115	31	530	18	150	625	25	2200	20.0	7.65	2.40	6.9	3.04
116	31	165	23	130	585	32	1900	24.0	5.25	2.26	6.5	2.98
119	33	155	25	130	650	35	3500	16.5	5.75	2.00	11.5	2.80
23	31	148	16	190	960	11	2500	17.5	4.60	1.85	12.5	3.82
24	31	440	20	200	480	3	1520	15.0	5.00	2.00	10.0	3.61
25	32	170	17	135	770	17	1650	24.5	6.00	3.00	10.8	4.34
26	31	120	24	143	455	9	2500	24.0	4.75	1.92	7.4	3.78
27	31	57	26	143	670	21	3050	22.5	2.70	1.51	7.8	3.55
214	31	530	18	150	600	69	4000	13.0	9.50	.55	7.8	2.02
215	31	390	18	165	1100	47	3750	20.5	7.50	1.71	9.6	3.65
31	31	90	22	165	1250	21	3750	22.5	4.75	2.16	6.3	2.85
32	32	65	15	138	850	13	2000	27.0	5.00	3.50	12.4	3.04
32	31	218	20	143	510	8	1900	21.5	4.00	1.99	10.5	3.16
34	31	250	21	155	455	8	2500	15.5	3.75	1.64	10.5	2.98
35	32	280	19	1600	2150	19	2500	23.5	5.50	2.15	13.4	3.57
35	31	260	26	165	585	9	2340	25.0	4.00	1.51	8.1	2.92
36	32	70	15	135	550	13	1750	23.0	4.75	2.00	12.6	3.10
36	31	57	23	110	585	7	1650	23.5	4.00	2.33	8.5	3.51
37	32	140	19	183	950	12	2150	21.5	5.50	2.25	17.4	3.29
37	31	65	24	95	585	4	1575	25.5	4.10	1.44	7.4	4.07
313	32	410	21	173	1100	17	2100	22.0	5.75	2.75	13.7	3.16
43	31	70	18	155	740	10	2340	21.5	3.90	2.33	8.9	3.20
44	32	170	15	113	610	11	2450	20.5	5.75	2.00	15.0	3.02
44	31	185	19	230	440	11	3275	15.5	5.50	.96	10.0	3.19
45	32	405	15	115	400	17	3000	23.0	6.75	1.50	12.6	3.28
46	31	157	25	155	625	10	3500	20.2	4.25	1.71	8.1	3.05
47	31	120	17	165	585	12	3050	18.0	3.70	2.54	9.6	3.36
411	31	110	17	143	455	10	2650	22.5	4.85	1.33	6.9	3.72
420	33	275	23	165	530	20	1750	20.0	10.25	1.00	15.0	2.71
51	31	500	24	275	720	13	2340	18.0	6.50	.80	10.5	2.71
52	31	175	24	165	390	10	1650	15.0	6.10	2.18	12.5	3.59
53	32	80	21	650	700	27	6400	21.5	5.00	1.70	12.6	3.41
53	31	120	21	95	545	10	2100	22.5	4.00	1.51	6.5	4.36
54	31	208	20	165	600	12	2200	22.5	5.00	2.40	4.6	3.91
55	32	115	15	105	490	16	3550	22.5	4.75	2.50	14.2	4.07

55	31	208	26	155	625	18	3500	18.5	4.10	3.00	8.9	2.91
57	32	90	17	90	590	8	3100	22.0	4.50	2.25	14.2	3.70
57	31	148	24	150	545	15	3500	20.0	5.00	1.92	7.8	3.78
59	31	128	14	150	455	10	3500	21.0	5.25	1.50	5.9	4.03
510	31	228	17	150	900	7	1800	18.0	3.10	1.16	8.9	2.90
511	32	275	15	175	500	10	1500	18.0	5.00	1.50	22.6	3.59
512	32	190	14	73	950	11	2650	18.5	6.00	.90	14.8	3.32
512	31	405	14	190	470	22	2650	19.5	6.85	.50	10.8	3.00
513	32	385	14	1400	570	29	4500	19.5	8.25	.50	15.3	3.89
513	31	240	20	165	490	15	2500	19.0	5.30	1.03	9.6	3.49
515	32	330	15	1875	150	70	1250	25.5	8.00	.50	11.1	3.91
61	31	120	17	150	600	11	2650	17.5	4.20	1.85	10.5	2.68
62	32	580	17	263	490	19	1950	22.0	8.55	1.00	13.7	3.26
62	31	138	25	115	455	18	3500	20.0	6.00	1.58	6.5	3.66
63	32	250	18	275	1100	15	2100	26.5	6.25	2.65	11.9	2.79
64	31	157	25	150	585	10	2340	24.5	5.50	1.71	5.9	2.73
67	32	55	15	250	950	15	3150	29.5	5.25	2.30	11.1	3.55
67	31	81	23	165	765	7	2000	27.5	4.75	3.08	5.5	2.73
610	32	110	15	110	850	18	1200	25.5	5.25	3.80	13.4	3.56
611	31	440	24	310	440	12	2340	16.5	5.40	.96	10.0	2.85
612	31	157	20	230	765	13	3500	15.5	5.50	.96	10.8	2.43
615	32	60	12	83	240	10	1050	21.5	6.75	1.00	12.9	3.72
616	31	198	25	150	330	15	2200	26.5	5.65	1.03	5.5	4.67
73	33	40	20	85	775	15	1200	13.0	4.25	1.70	11.2	4.61
75	33	105	25	115	650	15	1500	22.0	4.75	1.70	11.2	3.93
76	33	85	20	145	700	13	950	20.5	4.50	.60	15.7	2.82
77	33	90	20	125	590	15	1200	25.0	4.50	1.20	13.2	2.54
78	33	40	20	85	1350	18	1400	22.0	3.25	2.20	12.3	3.59
720	13	40	18	50	300	10	750	18.0	2.75	.85	7.7	6.72

WEINMANNIA RACEMOSA LEAVES

SITE NO.	NI PPM	CO PPM	CU PPM	ZN PPM	CR PPM	FE PPM	CA PCENT.	MG PCENT.	MN PCENT.	K PCENT.	ASH PCENT.
11	202	17	81	159	60	525	21.4	10.00	1.10	7.8	4.42
12	88	15	63	333	10	500	23.4	8.00	1.40	7.8	4.52
13	105	13	81	188	75	595	23.8	8.50	1.15	8.6	4.52
14	145	13	81	250	35	525	24.4	8.50	2.05	9.0	5.42
15	500	11	68	238	40	780	22.6	7.50	1.70	10.5	3.34
16	125	8	124	448	10	965	11.6	4.75	.80	6.4	3.49
17	63	14	57	238	20	550	27.4	6.00	1.40	6.4	5.39
18	70	14	53	283	65	1520	23.8	8.75	1.85	8.6	3.91
19	125	13	81	133	360	750	22.6	11.00	.95	9.0	3.89
110	105	7	68	168	175	595	12.8	7.00	.40	2.7	3.60
111	96	14	41	216	320	925	23.8	8.50	1.65	6.0	6.22
112	135	15	46	153	215	480	25.0	7.75	.95	7.1	6.28
113	145	11	75	228	200	640	22.6	11.50	1.35	6.0	4.81
114	135	12	52	333	175	410	25.0	10.50	1.25	3.8	5.50
115	300	15	63	250	250	615	23.2	12.75	1.40	4.5	4.72
116	410	15	57	125	700	525	21.4	14.75	.70	5.0	4.85
21	105	19	75	400	30	570	25.0	9.25	1.25	6.4	5.45
22	125	13	68	394	23	780	22.0	10.00	1.45	7.1	4.39
23	105	10	75	150	23	570	21.4	9.00	1.80	8.6	3.85
24	190	12	81	198	83	570	22.6	10.75	2.00	6.4	5.08
25	105	15	81	216	23	1375	22.0	10.50	.95	8.6	4.57
26	41	11	87	228	15	850	20.7	10.75	1.45	14.8	5.85
27	41	14	68	250	10	640	24.4	4.75	2.05	10.5	5.64
29	125	16	68	178	200	1010	22.6	9.75	1.20	7.5	3.94
210	250	20	81	159	305	850	27.4	10.75	1.55	6.8	4.50
211	125	11	75	188	100	640	23.2	16.50	1.05	6.8	3.68
212	178	18	130	150	110	430	23.2	8.25	1.40	9.0	4.27
213	375	14	63	118	550	700	20.7	14.50	.90	6.4	4.18
214	145	18	68	160	450	750	19.5	16.25	1.45	7.5	3.95
215	155	14	63	150	240	725	23.2	11.75	1.10	5.0	4.95
216	166	17	52	271	285	1150	25.6	8.75	1.20	6.4	5.04
31	48	12	81	320	10	815	23.8	7.25	2.60	11.2	4.72
32	105	14	93	238	35	595	18.3	7.00	2.70	16.3	3.28
33	145	14	87	283	45	700	21.3	7.50	1.95	10.8	4.69
34	145	16	57	260	40	1150	23.8	8.50	1.00	9.0	4.78
35	63	12	130	271	10	1300	20.7	8.25	1.00	15.2	4.35
36	63	13	57	216	25	550	23.8	7.50	2.00	9.0	4.16
37	51	14	63	216	15	850	23.8	10.25	1.85	6.8	5.17
38	125	8	46	308	65	500	21.3	9.25	2.55	6.0	6.31
39	190	32	63	238	430	750	22.6	12.00	1.15	5.3	4.60
310	96	15	87	207	115	525	22.6	11.00	1.15	8.2	4.04
311	190	15	75	178	350	500	23.8	11.25	.90	6.4	4.40

312	225	12	52	178	205	725	18.9	12.25	.90	5.7	4.79
313	155	11	99	250	105	640	23.2	10.25	1.15	9.0	6.01
314	145	16	46	168	225	750	26.2	8.25	1.60	5.3	5.69
315	96	17	81	216	130	625	23.8	6.75	1.25	8.9	5.44
316	178	16	63	168	510	640	21.9	8.75	.60	7.4	5.17
41	88	11	110	420	40	1375	18.3	10.50	2.30	14.2	4.27
42	79	14	87	283	88	1200	19.5	8.75	2.20	9.3	4.53
43	51	12	87	238	65	665	22.6	6.25	2.15	11.5	3.85
44	105	10	75	179	95	925	21.3	6.75	1.50	13.0	4.46
45	145	14	75	250	23	850	21.3	8.00	1.70	12.0	3.87
46	63	12	57	178	10	880	23.2	7.25	1.75	8.6	5.22
47	79	15	81	333	18	2350	20.1	8.50	2.35	9.3	4.43
48	70	14	124	260	25	465	23.8	8.75	1.90	9.0	3.93
49	166	17	46	308	68	400	23.2	9.00	1.85	7.5	1.27
410	79	13	57	207	25	925	21.3	10.00	1.20	7.8	4.64
411	105	14	75	143	45	410	20.1	8.75	1.00	11.2	4.60
412	166	17	87	283	85	2150	23.8	8.75	.60	5.3	4.13
413	105	15	57	198	255	410	22.6	11.00	.90	6.2	7.11
414	105	16	41	216	265	525	23.2	11.00	1.40	4.5	4.62
415	250	13	46	159	740	700	23.8	9.50	.75	11.2	5.08
416	135	16	68	216	135	700	21.9	12.25	1.15	7.1	4.28
52	201	16	68	216	190	640	23.1	7.25	1.15	9.0	4.47
53	105	17	46	178	63	570	23.8	7.25	.90	9.6	5.43
54	63	13	99	308	5	1250	24.4	5.50	2.00	2.3	6.04
55	115	9	63	283	35	570	21.3	7.00	1.90	12.6	4.99
57	63	13	68	207	18	1700	19.5	6.50	1.90	10.7	5.38
58	70	14	57	125	58	550	21.3	10.00	.75	8.6	4.63
56	51	13	99	207	20	525	21.9	7.50	1.70	11.2	4.90
59	51	16	75	271	40	2350	19.5	9.00	1.10	9.3	8.40
510	178	13	75	178	185	700	20.1	7.25	.95	12.0	4.40
511	88	10	99	271	45	1700	20.1	9.75	2.00	9.6	4.20
512	264	16	124	188	400	965	21.9	8.25	.60	8.2	4.54
513	315	13	130	260	125	880	21.9	10.50	.95	8.2	3.83
514	88	11	68	150	83	1010	18.3	12.75	.90	8.6	3.63
515	70	13	81	143	125	725	21.9	9.50	.65	8.6	4.70
516	225	20	52	143	390	615	21.3	10.75	1.20	6.8	4.88
61	70	10	75	343	15	925	18.3	8.25	1.65	15.5	4.45
62	125	18	63	216	190	780	22.6	9.75	.75	11.2	5.59
63	115	19	41	216	90	525	24.4	10.25	1.00	5.7	4.91
65	70	10	87	250	10	1050	18.3	5.05	1.25	11.5	4.85
66	88	11	52	207	35	1375	23.2	7.25	1.70	10.0	5.02
67	48	12	41	159	23	500	23.8	6.00	1.50	7.8	6.20
68	63	12	52	295	20	665	21.3	6.75	1.90	10.8	4.80
69	48	12	68	320	10	665	22.6	7.75	2.05	8.2	4.68
610	48	12	52	228	28	500	22.6	9.75	.75	9.3	4.95
611	125	11	93	250	13	1150	27.7	4.75	1.40	10.8	5.45
612	166	15	52	238	20	780	17.7	11.00	1.90	5.3	4.33
613	48	14	46	198	13	550	25.6	6.25	1.05	7.1	6.03

614	96	15	93	338	80	700	22.6	9.00	1.20	12.3	3.72
615	213	12	81	95	440	1600	19.5	9.00	.60	10.5	5.12
616	125	15	81	188	98	1375	21.3	10.00	1.00	6.8	4.25

QUINTINIA ACUTIFOLIA LEAVES

SITE NO.	NI	CO	CU	ZN	CR	FE	CA	MG	MN	K	ASH
	PPM	PPM	PPM	PPM	PPM	PPM	PCENT.	PCENT.	PCENT.	PCENT.	PCENT.
11	75	16	50	185	14	730	17.0	6.25	1.65	23.9	5.10
12	94	17	67	185	10	1080	18.5	4.50	2.15	26.1	5.18
13	162	18	80	223	33	850	21.0	8.75	1.05	17.2	5.00
14	128	18	89	185	14	630	16.5	8.25	1.70	22.2	5.20
15	64	16	96	185	23	955	13.0	4.50	2.65	29.4	5.85
16	69	16	96	223	9	730	14.5	4.75	1.90	27.8	6.62
17	62	17	64	200	13	1850	18.5	6.00	1.85	21.6	6.37
18	94	18	87	223	29	2100	19.5	9.75	1.35	16.7	4.84
19	121	17	81	210	26	955	18.0	7.25	2.15	21.6	4.72
110	84	16	48	150	110	580	22.0	11.25	1.25	14.5	4.82
111	68	15	56	175	40	920	17.5	6.75	3.50	18.3	5.86
112	79	18	63	223	17	730	22.5	6.75	2.30	14.5	5.67
113	108	17	53	133	64	850	23.0	11.50	1.80	8.9	6.51
114	116	20	72	150	21	580	23.0	7.25	1.40	15.6	5.74
115	108	18	48	200	32	920	22.0	12.00	1.60	10.5	5.50
116	128	19	60	133	33	550	22.0	12.25	1.65	10.5	5.63
21	110	17	75	200	10	1275	18.5	9.00	1.85	18.9	4.86
22	87	15	57	150	17	850	17.0	7.25	2.75	20.6	5.51
23	105	15	59	175	12	850	16.0	5.50	2.65	22.8	4.28
24	106	17	69	295	15	3500	20.5	9.00	2.80	10.0	5.34
25	80	17	84	185	11	850	19.0	6.75	2.15	18.9	5.39
26	57	17	91	210	12	1225	20.5	6.00	1.80	17.2	6.07
27	33	15	74	133	10	730	18.0	5.00	1.75	21.1	5.40
28	225	20	234	185	28	4000	21.0	7.50	1.50	10.0	6.28
29	88	17	87	200	37	820	23.0	7.50	2.15	15.0	4.46
210	89	19	74	210	11	1045	21.5	6.25	1.85	18.3	5.02
211	104	18	63	223	19	850	21.5	13.25	1.10	10.0	5.05
212	128	17	45	165	19	1225	17.0	8.00	2.05	22.2	5.07
213	121	16	72	223	74	920	15.0	11.50	3.90	18.3	4.24
214	66	16	58	133	20	850	18.0	5.25	5.00	21.1	5.66
215	165	16	67	223	105	630	20.0	13.00	2.25	13.9	4.56
216	123	16	75	250	45	3000	18.5	8.25	2.00	17.2	5.73
32	70	15	124	210	29	655	15.5	6.00	2.50	27.2	4.40
33	162	15	54	165	17	850	15.0	6.00	1.60	23.4	5.59
34	72	17	66	223	15	2750	19.5	5.50	2.35	21.7	5.35
35	60	18	72	210	11	890	18.5	5.75	2.20	23.4	5.38
36	40	18	74	150	11	920	16.5	6.75	1.60	22.2	5.08
37	69	18	88	200	10	705	14.0	4.75	2.65	27.8	6.61
38	97	16	63	133	34	850	15.0	7.00	1.85	22.8	4.79
39	106	19	90	185	27	755	18.0	9.25	1.35	21.1	4.44
310	61	19	70	175	12	1850	19.0	6.75	1.70	22.8	5.15
311	89	19	60	175	66	1225	19.0	13.50	1.70	15.6	4.47

312	146	19	75	185	60	1330	20.0	11.50	1.75	13.9	5.50
313	90	18	75	185	20	890	23.0	8.25	2.85	13.9	5.31
314	104	19	53	200	65	655	24.0	9.25	4.20	9.5	5.99
315	81	18	52	150	15	755	19.0	6.75	1.85	20.6	5.63
316	99	20	57	175	13	1125	21.0	6.75	2.10	18.9	5.60
41	62	16	94	185	12	1680	15.0	6.75	2.20	23.4	6.16
42	117	18	81	295	15	1680	17.5	4.75	2.85	19.5	5.40
44	132	19	89	250	17	580	24.5	8.50	1.85	10.0	5.11
45	87	18	45	250	10	730	27.5	7.50	2.45	8.9	5.89
46	60	17	57	250	13	955	23.5	6.00	3.50	13.9	6.45
47	43	16	74	235	15	1465	17.5	3.50	6.75	18.3	6.07
48	80	18	63	150	12	630	26.5	6.50	2.15	12.8	6.12
49	72	17	89	165	21	705	22.0	7.25	3.25	13.3	6.87
410	56	17	58	200	11	705	22.5	7.50	2.70	15.0	5.76
411	55	17	75	223	14	705	20.5	5.50	1.30	20.6	6.06
413	82	16	63	143	85	705	18.0	11.00	.65	18.9	5.09
414	128	15	53	175	70	850	21.5	11.00	1.85	13.3	4.63
415	83	16	94	210	65	755	16.5	9.50	3.10	18.9	5.35
416	138	17	73	150	29	630	22.5	11.00	1.45	13.9	5.15
51	175	16	69	223	212	1700	20.0	10.25	1.25	13.3	5.25
52	104	17	89	165	14	850	18.5	6.00	2.75	21.6	6.73
53	79	15	145	250	20	1040	16.3	7.80	6.25	25.0	4.38
54	92	17	80	295	14	1850	20.0	8.50	2.85	12.4	5.38
55	83	16	68	210	14	560	19.0	5.50	7.10	16.1	6.01
56	64	17	64	200	13	850	21.5	3.50	5.25	17.2	5.34
57	95	20	325	223	14	1850	18.5	6.50	1.70	19.5	5.35
59	43	18	73	150	13	955	19.5	6.75	.95	21.6	5.80
511	104	16	110	175	14	1225	17.5	6.50	2.85	21.6	5.24
512	119	16	42	115	84	890	20.5	8.75	1.05	18.9	6.12
513	106	21	63	225	26	1550	24.3	11.50	1.02	14.2	4.92
514	109	19	79	185	39	1850	17.0	7.75	2.80	20.0	6.74
515	72	17	60	200	11	1975	18.0	7.25	.85	23.4	5.34
516	270	19	81	133	59	730	20.5	13.50	2.50	12.8	4.75
61	77	16	59	210	12	1275	17.0	5.75	2.25	25.6	5.31
62	70	17	79	235	13	1600	18.0	7.50	2.35	18.3	5.14
63	87	17	85	280	9	790	21.0	9.50	1.50	15.0	5.00
64	72	15	48	133	12	655	16.5	7.00	2.30	23.4	5.96
65	75	16	65	133	10	580	17.0	4.50	1.65	27.2	5.51
66	79	15	134	263	15	3850	19.5	8.25	2.10	15.6	5.16
67	63	17	69	210	12	1175	21.5	6.00	1.75	16.7	5.66
68	52	17	60	165	13	920	18.5	4.75	3.40	23.4	5.69
69	64	15	65	143	11	1125	19.0	5.50	5.00	17.8	5.33
610	62	16	53	210	13	655	20.5	7.00	2.30	17.8	5.46
612	92	17	66	200	15	3000	20.0	8.00	2.46	20.0	5.52
613	115	17	70	210	20	705	19.5	7.75	1.90	19.5	4.21
618	185	16	142	295	85	4000	15.5	11.50	.35	22.8	3.78

QUINTINIA ACUTIFOLIA TWIGS

SITE	NI PPM	CU PPM	ZN PPM	FE PPM	ASH PCNT.
11	41	124	600	455	.98
12	42	64	150	215	2.33
13	77	177	280	415	.93
14	67	130	850	440	.82
15	59	210	550	400	1.46
16	77	84	1025	265	1.46
17	52	80	370	300	1.67
18	39	99	280	315	1.95
19	57	89	1373	470	1.69
110	52	99	850	250	.89
111	41	80	435	300	1.63
112	37	115	85	225	7.15
113	69	105	550	300	1.00
114	78	136	990	455	1.09
115	54	99	1025	350	1.71
116	62	110	2250	455	.93
21	42	118	940	315	1.58
22	31	99	625	265	1.39
23	52	118	430	655	1.69
24	71	99	1500	265	.96
25	61	130	370	1225	1.61
26	46	124	500	2100	1.39
27	31	150	765	390	1.11
28	126	285	475	4000	1.76
29	48	132	430	390	1.16
210	43	105	1350	400	1.23
211	75	122	890	565	1.12
212	59	92	940	350	1.53
213	56	96	475	350	1.76
214	38	64	850	250	1.99
215	122	114	1390	730	.90
216	72	130	295	565	1.20
32	58	134	500	1080	1.28
33	96	122	890	540	1.13
34	48	80	940	340	1.51
35	42	217	1150	415	1.28
36	32	122	430	200	1.51
37	53	99	890	300	2.99
38	47	107	233	580	1.09
39	106	212	394	415	.81
310	42	120	800	300	1.21
311	64	160	305	700	1.41

WEINMANNIA RACEMOSA TWIGS

SITE	NI PPM	CU PPM	ZN PPM	FE PPM	ASH PCNT.
11	75	95	300	650	2.10
12	50	130	430	500	1.66
13	63	165	380	620	1.00
14	55	100	250	420	2.12
15	55	210	400	1450	.84
16	55	185	600	400	.93
17	28	140	200	300	1.51
18	68	130	210	1350	1.51
19	75	196	300	900	1.15
110	55	140	360	650	.94
111	63	120	330	420	1.71
112	75	225	250	1050	2.08
113	75	130	410	620	1.41
114	55	80	300	500	1.82
115	123	80	220	490	2.40
116	265	115	210	450	1.58
21	63	145	450	620	1.57
22	75	165	430	1780	1.12
23	63	160	250	900	1.97
24	75	240	280	720	.83
25	55	210	320	770	1.25
26	28	200	270	500	1.13
27	21	95	200	610	2.25
28	335	250	210	2650	1.31
29	55	185	450	1500	.81
210	84	140	225	999	.97
211	55	115	210	655	.73
212	130	195	235	999	1.77
213	470	140	245	690	1.42
214	84	100	270	770	1.73
215	90	160	410	1100	1.53
216	63	145	290	750	.96
31	28	125	260	460	2.09
32	68	225	450	770	1.54
33	50	270	500	1200	.90
34	84	180	280	1350	1.26
35	28	270	400	1650	.99
36	15	180	270	770	7.45
37	28	215	430	820	1.12
38	90	155	630	655	4.39
39	50	185	380	420	1.15
310	40	130	225	650	1.33

NOTHOFAGUS SPP. TWIGS

SITE	NI PPM	CU PPM	ZN PPM	FE PPM	ASH PCNT.
11	350	75	365	1140	2.46
13	420	113	150	450	1.30
14	88	113	450	920	1.25
15	125	93	125	275	1.52
17	154	93	155	520	2.74
18	144	69	250	420	2.21
19	63	69	235	430	1.75
110	152	83	120	533	1.81
111	33	46	90	320	3.47
112	125	69	130	420	2.96
113	106	58	75	400	2.78
114	54	46	275	920	3.55
115	422	92	486	1334	3.23
116	63	63	310	420	3.24
21	63	69	310	1145	3.06
22	106	63	125	640	2.29
23	54	46	310	200	3.29
24	153	77	235	533	4.00
26	79	106	350	1400	3.46
27	29	58	450	490	3.86
28	405	300	250	1350	1.29
29	106	63	140	550	3.54
210	63	46	80	250	2.26
211	63	51	160	1195	1.98
212	164	58	125	875	1.81
213	71	69	140	550	1.96
214	218	141	1032	2065	.69
215	320	106	500	450	1.58
216	97	58	100	370	4.14
31	97	93	500	625	1.71
32	106	75	425	720	1.82
34	71	46	250	275	3.92
35	97	51	310	470	2.27
36	29	63	340	310	1.81
37	31	35	240	290	4.46
38	33	75	130	310	2.72
311	300	93	240	275	1.57
312	54	51	110	320	1.74
314	300	93	110	320	1.64
316	164	75	105	450	2.28
41	49	61	133	533	1.59
43	88	113	570	1140	1.54

312	63	113	400	400	1.65	311	90	115	235	470	1.36	44	144	159	375	2325	2.70
313	27	84	430	350	1.86	312	68	115	225	1350	1.44	45	94	132	400	2566	4.17
314	62	73	330	225	1.92	313	106	150	410	690	1.43	46	79	132	450	1250	1.95
315	40	136	1350	390	1.09	314	73	220	330	720	1.26	47	144	75	470	470	1.71
316	42	110	1025	520	1.20	315	35	185	210	1100	1.74	48	54	93	110	420	2.44
41	44	156	370	340	1.32	316	100	195	190	900	1.22	49	88	69	110	670	1.48
42	67	200	990	2350	1.20	41	55	300	525	1050	.73	410	106	69	125	450	1.48
44	75	177	430	365	1.34	42	35	195	300	1350	1.38	411	54	46	240	670	4.14
45	54	74	950	530	1.62	43	28	230	360	1400	1.18	413	250	75	125	640	1.83
46	33	88	1035	475	3.46	44	63	180	250	1620	1.75	414	195	51	80	640	2.28
47	31	117	1350	455	1.29	45	63	165	290	1025	1.66	416	83	231	333	654	1.24
48	39	130	575	655	1.19	46	35	150	245	770	1.13	51	680	174	450	2070	.89
49	44	99	1350	390	1.44	47	35	145	290	2100	1.56	52	71	58	85	490	2.10
410	32	124	1090	340	.97	48	68	210	330	900	1.14	53	88	51	225	400	3.07
411	42	99	410	250	1.59	49	100	165	260	400	1.19	54	154	125	340	2050	4.30
413	42	143	475	250	.81	410	40	335	250	720	.74	55	115	75	275	1400	2.91
414	60	88	1850	315	1.42	411	55	200	180	420	.97	56	300	63	120	275	2.18
415	29	94	350	320	1.67	412	106	180	280	1500	.81	57	134	51	170	640	2.38
416	63	134	800	400	1.08	413	68	185	210	850	1.20	58	106	113	150	520	2.59
51	132	105	313	400	1.59	414	63	125	250	720	6.21	59	217	159	365	920	1.17
52	83	150	625	655	1.78	415	115	165	245	820	.85	510	144	93	450	920	1.61
53	32	156	313	390	1.19	416	63	55	115	280	2.13	512	545	99	320	670	1.43
54	65	118	660	630	1.10	52	90	165	225	820	1.72	513	217	99	300	875	2.29
55	52	96	450	225	1.51	53	55	210	200	1100	1.24	515	94	61	100	426	2.77
56	46	92	990	225	1.57	54	63	70	90	500	6.70	516	250	131	365	999	3.87
57	59	141	1090	1400	1.15	55	55	115	320	770	3.35	61	79	75	470	1040	1.87
59	48	84	175	455	1.92	56	40	210	430	655	1.09	62	217	99	365	2090	1.70
511	62	22	210	475	1.74	57	63	200	430	1550	1.64	64	88	93	385	1350	1.69
512	54	92	313	390	.83	58	55	125	150	1025	1.61	65	97	80	140	670	2.37
513	98	118	1150	1850	2.42	59	35	175	280	1050	1.73	67	79	113	470	490	1.92
514	40	150	999	1460	1.37	510	68	210	150	655	1.79	68	54	69	150	380	1.63
515	49	105	475	440	1.67	511	75	230	485	999	1.61	69	49	254	385	1140	1.65
516	82	136	1025	315	1.93	512	75	145	190	820	1.30	611	261	159	350	690	1.63
61	32	105	800	520	1.36	5132	140	300	280	1630	1.28	612	362	221	920	2066	.89
62	42	92	940	200	1.22	514	68	105	150	770	1.08	6125	106	58	140	520	2.48
63	62	177	890	2200	1.02	515	40	160	250	770	1.66	613	115	80	240	550	1.69
64	39	80	450	225	2.48	516	130	185	245	128	1.09	614	261	99	225	5500	1.82
65	27	92	695	190	1.50	61	40	210	450	600	1.14	615	144	69	155	3500	4.36
66	62	96	223	705	1.58	62	68	175	290	950	1.14	616	154	106	300	1475	3.13
67	50	88	1275	300	1.49	63	63	150	290	820	1.24	617	327	70	400	940	1.94
68	42	69	990	350	2.31	65	123	50	150	500	1.67	618	300	131	190	750	1.74
69	30	69	350	215	2.04	66	55	150	270	1200	1.92	619	446	141	213	999	1.47
610	33	60	1150	250	1.19	67	28	215	290	500	.93	0	0	0	0	0	0.00
612	40	135	1320	555	1.18	68	21	165	350	850	1.55	0	0	0	0	0	0.00
613	47	173	410	630	1.69	69	35	145	400	750	1.13	0	0	0	0	0	0.00
618	113	173	223	475	1.40	610	28	150	500	650	1.17	0	0	0	0	0	0.00
0	0	0	0	0	0.00	611	90	90	120	350	2.29	0	0	0	0	0	0.00
0	0	0	0	0	0.00	612	63	165	290	1050	1.79	0	0	0	0	0	0.00

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0    0    0    0    0  0.00  6125   75  145  270   900  1.88    0    0    0    0    0  0.00
0    0    0    0    0  0.00   613   35  100  135   655  2.02    0    0    0    0    0  0.00
0    0    0    0    0  0.00   614  100  160  160   460  2.23    0    0    0    0    0  0.00
0    0    0    0    0  0.00   615   68  165  150  1850  1.61    0    0    0    0    0  0.00
0    0    0    0    0  0.00   616   84  200  245  1850  1.44    0    0    0    0    0  0.00
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SOILS

IDENTIFICATION	-20 MESH	-120 MESH	-400 MESH	HCL EXTRACT
MARCH 1969	11	21	31	41
NOVEMBER 1969	12	22	32	42
NOVEMBER 1970	13	23	33	43

SOILS -20 MESH

SITE NO.	IDENT.	NI PPM	CO PPM	CU PPM	ZN PPM	CR PPM	CA PCENT.	MG PCENT.	MN PCENT.	K PCENT.	PH
10	13	285	125	240	100	925	8.0	8.7	.155	.38	5.50
11	11	59	36	45	51	205	1.7	1.9	.053	1.23	4.86
11	12	80	36	52	76	301	2.2	2.4	.060	.52	5.25
12	11	45	28	24	49	174	1.9	1.5	.069	.11	4.89
13	11	298	77	194	113	800	6.7	6.8	.135	.57	6.53
13	12	176	67	180	114	510	6.0	5.6	.137	.90	5.75
14	11	180	68	146	131	409	3.9	3.8	.148	.37	5.54
14	12	248	75	251	131	563	6.1	5.2	.161	.60	5.80
15	11	114	51	37	86	396	5.2	3.7	.110	.71	5.32
15	12	146	58	30	88	632	6.5	5.7	.123	.69	4.55
16	11	108	67	40	82	304	5.0	3.0	.121	.51	5.46
16	12	117	63	87	113	338	5.4	4.1	.165	.75	5.85
17	11	142	71	94	107	336	4.6	3.3	.189	.40	6.26
17	12	201	62	50	77	772	9.2	7.7	.139	.50	6.15
18	11	219	62	26	69	1068	7.1	6.2	.115	.24	6.00
18	12	208	50	15	73	1428	8.3	7.9	.116	.46	5.50
19	11	161	50	40	75	552	5.3	4.4	.087	.49	5.08
19	12	164	49	41	82	900	6.7	6.1	.094	.61	5.90
110	11	200	67	23	79	1219	6.9	6.1	.147	.34	6.02
110	12	160	56	24	68	880	7.2	6.0	.116	.72	5.80
111	11	204	54	18	63	1305	6.7	6.1	.094	.24	5.35
111	12	186	47	14	74	1284	7.7	6.7	.105	.47	5.55
112	11	147	42	34	49	680	4.6	4.6	.080	.27	4.45
112	12	152	44	47	71	809	5.3	5.0	.084	.61	4.70
113	11	265	56	50	90	1269	7.7	7.0	.112	.19	5.76
113	12	285	53	56	73	1290	8.6	7.9	.113	.36	5.30
114	11	205	60	140	77	816	6.2	5.6	.100	.41	5.00
114	12	246	27	230	77	1099	7.9	8.3	.107	.13	4.80
115	11	181	51	45	60	728	6.1	5.4	.094	.45	5.40
115	12	217	50	68	71	959	6.9	6.7	.103	.50	4.70
116	11	196	57	42	74	897	4.8	4.3	.087	.69	5.16
116	12	204	55	36	95	946	5.4	5.1	.087	.76	5.20
117	12	182	55	20	78	1105	6.9	5.5	.104	.81	5.30

118	13	290	75	20	75	1350	7.8	7.3	.120	.27	5.15
119	13	750	185	35	115	1750	5.9	6.5	.275	.16	6.55
120	13	285	70	30	80	1100	7.0	7.0	.115	.28	5.60
20	13	50	35	20	75	125	.8	1.0	.050	1.68	4.50
21	11	133	58	126	71	336	4.8	3.7	.092	.31	5.26
21	12	198	70	246	118	512	8.8	4.8	.102	.51	5.50
22	11	120	60	68	74	290	5.6	4.2	.103	.48	4.90
22	12	159	70	119	93	430	6.9	5.6	.119	.66	5.55
23	11	229	68	40	220	845	5.3	6.3	.129	.42	5.20
23	12	356	78	146	136	1005	5.5	7.1	.123	.52	5.50
24	11	257	68	205	107	552	4.2	4.5	.106	.36	5.44
24	12	266	70	67	113	966	5.8	7.3	.139	.47	5.30
25	11	179	62	67	92	607	6.3	5.0	.139	.48	5.30
25	12	146	54	48	86	668	6.9	6.0	.149	.64	5.80
26	11	117	59	84	80	300	4.6	3.2	.108	.34	5.40
27	11	153	69	131	103	456	5.0	4.1	.130	.60	5.52
28	11	385	103	962	99	414	6.6	6.2	.170	.34	5.90
29	11	236	64	40	62	1448	9.0	6.8	.123	.25	5.80
29	12	228	61	14	78	1424	8.9	7.4	.125	.61	5.40
210	11	199	58	30	75	1139	6.5	5.2	.106	.44	5.32
210	12	224	62	24	93	1311	7.7	6.5	.117	.59	5.40
211	11	193	55	21	66	1092	6.4	5.0	.085	.36	6.16
211	12	176	49	13	75	1401	8.1	6.5	.111	.55	5.75
212	11	322	62	40	83	897	4.8	4.1	.087	.54	4.92
212	12	220	57	40	94	1202	6.0	5.6	.087	.67	5.50
213	11	198	53	23	57	1564	6.9	5.4	.105	.34	5.50
213	12	206	49	10	70	2099	8.8	7.5	.108	.53	5.55
214	11	239	93	14	62	1720	7.9	6.2	.181	.25	5.95
214	12	256	77	6	74	2112	9.2	8.3	.163	.42	5.50
215	11	255	72	64	72	1209	6.5	5.4	.125	.29	5.73
215	12	238	65	14	78	1326	9.1	7.4	.136	.78	5.50
216	11	289	74	68	90	1057	4.7	4.5	.112	.38	5.72
216	12	116	39	53	85	422	2.9	2.8	.063	.99	5.45
217	12	356	102	68	132	1017	5.0	5.4	.163	.92	5.35
218	13	250	80	30	85	1150	7.3	6.2	.130	.45	5.45
219	13	280	85	25	80	1550	9.7	7.8	.155	.25	6.10
220	13	230	65	35	75	1050	7.7	6.5	.105	.40	5.35
30	13	75	45	45	70	175	1.6	1.7	.060	1.10	4.80
31	11	64	41	41	73	188	1.1	1.4	.056	1.30	4.85
32	11	108	61	122	67	283	3.8	2.6	.072	.77	5.70
32	12	97	59	138	83	294	4.4	3.4	.086	.97	5.45
33	11	112	59	154	84	330	4.4	3.0	.101	.75	5.50
34	11	260	76	82	99	826	5.6	6.7	.126	.41	5.43
34	12	210	76	115	118	559	5.2	5.2	.135	.59	5.40
35	11	108	65	96	92	243	5.0	3.7	.145	.47	5.60
35	12	109	72	89	109	239	5.3	4.1	.130	.68	5.35
36	11	77	68	63	103	162	5.5	3.7	.140	.42	5.20
36	12	138	69	56	125	395	6.0	4.6	.174	.69	5.50

37	11	30	34	17	39	87	3.0	1.1	.082	.60	6.13
37	12	123	65	26	97	388	7.4	5.4	.145	.61	4.60
38	11	148	70	83	80	369	4.1	4.0	.165	.55	5.84
38	12	181	91	106	110	604	5.5	6.0	.226	.96	5.60
39	11	177	75	88	73	559	6.8	6.1	.167	.46	4.98
310	11	164	76	95	89	400	6.2	5.5	.120	.56	5.06
311	11	213	66	106	70	489	8.6	7.2	.115	.33	5.25
311	12	237	84	227	79	542	7.8	6.9	.153	.48	6.10
312	11	206	62	37	70	943	6.6	5.7	.092	.49	5.50
312	12	253	71	36	96	1063	6.0	6.0	.111	.76	5.70
313	11	114	42	16	58	567	5.0	3.9	.109	.58	5.96
313	12	136	49	44	78	731	4.3	8.7	.093	1.02	5.35
314	11	239	64	64	87	801	4.4	4.2	.102	.71	4.86
314	12	192	58	19	72	1154	6.0	5.2	.111	.77	5.05
315	11	111	44	58	59	465	3.2	2.7	.064	.69	4.80
315	12	92	37	28	60	459	2.7	2.7	.060	.83	5.15
316	11	104	45	48	65	331	3.0	2.5	.055	.83	5.43
316	12	125	53	53	81	527	3.8	3.3	.077	1.25	5.30
317	13	160	60	45	75	850	4.2	3.5	.075	.80	5.95
318	13	250	85	50	90	1200	7.1	6.2	.125	.40	5.70
319	13	650	130	55	100	1600	5.4	6.5	.165	.33	6.00
320	13	310	80	40	85	1150	4.4	4.1	.080	1.05	5.35
40	13	60	40	35	75	150	.6	1.0	.050	1.38	4.40
41	11	83	39	79	79	206	1.5	1.6	.050	1.08	4.08
41	12	25	30	25	76	126	.7	1.5	.066	1.87	5.10
42	11	52	33	39	57	186	1.6	1.6	.070	1.07	4.58
43	11	48	29	24	40	146	1.3	1.2	.057	1.06	4.10
43	12	24	29	33	67	143	.9	1.4	.057	1.62	4.85
44	11	178	76	159	87	422	5.7	5.2	.137	.62	5.20
44	12	115	65	92	74	415	5.3	4.6	.129	.74	5.30
45	11	132	72	97	91	365	5.5	4.5	.150	.46	5.06
45	12	275	98	177	89	532	5.3	5.7	.173	.62	5.85
46	11	108	59	73	112	279	5.6	4.2	.175	.48	5.68
46	12	159	73	45	113	159	4.9	4.0	.159	.68	5.15
47	11	102	65	55	88	287	4.7	4.0	.151	.44	4.98
47	12	139	79	65	97	508	6.0	5.0	.139	.74	5.30
48	11	151	80	123	104	450	5.8	5.0	.153	.38	5.64
48	12	190	71	76	100	476	5.9	4.7	.143	.57	5.05
49	11	196	71	605	92	623	6.0	5.7	.133	.42	4.80
49	12	179	72	143	94	672	5.8	5.8	.144	.63	5.60
410	11	103	57	51	57	348	5.7	4.1	.114	.57	4.60
410	12	41	75	112	83	621	5.1	5.8	.136	.70	5.55
411	11	49	40	40	51	136	3.1	2.0	.097	.97	4.90
411	12	199	43	30	52	195	3.4	2.5	.099	1.34	5.75
412	11	266	94	220	87	744	6.7	6.5	.163	.46	6.25
413	11	269	122	459	69	887	9.7	7.9	.232	.14	6.50
413	12	280	88	66	66	964	10.9	7.8	.171	.35	6.20
414	11	229	84	42	88	728	6.5	6.2	.122	.43	5.76

414	12	215	107	40	98	805	6.7	6.7	.156	.63	5.65
415	11	207	72	32	68	1032	9.8	7.5	.144	.25	5.48
415	12	117	72	18	67	1210	9.4	7.5	.130	.54	5.90
416	11	130	59	134	64	523	4.7	4.0	.077	.81	5.58
416	12	132	57	47	67	615	4.7	4.2	.090	1.09	5.20
417	13	225	75	20	80	600	6.5	5.9	.110	.65	5.30
418	13	190	80	20	95	650	7.0	6.0	.115	.28	5.35
419	13	360	125	25	90	2000	6.4	7.2	.180	.25	5.70
420	13	260	75	40	85	1100	4.0	3.4	.075	1.38	5.75
50	13	105	60	50	150	200	.9	1.5	.070	1.68	4.60
51	11	80	57	65	64	345	4.6	3.8	.101	.64	4.62
51	12	277	65	165	61	346	3.8	3.4	.121	.78	5.00
52	11	201	92	201	87	598	6.6	5.7	.142	.44	5.42
52	12	141	100	341	109	500	5.0	5.4	.164	.82	5.05
53	11	133	79	90	75	440	5.7	5.2	.110	.37	5.39
53	12	202	82	46	78	733	6.8	6.8	.160	.64	5.30
54	11	182	71	76	83	333	6.6	5.7	.178	.33	5.69
54	12	193	91	180	86	386	6.0	5.5	.142	.69	5.90
55	11	172	67	70	84	643	8.3	7.2	.171	.28	5.48
55	12	146	87	114	91	546	6.3	6.3	.132	.73	5.30
56	11	296	92	333	132	430	4.9	4.7	.141	.46	5.20
56	12	345	75	115	93	354	4.8	4.8	.111	.62	5.20
57	11	112	69	155	123	210	5.2	3.3	.130	.40	4.72
57	12	152	123	493	90	658	6.9	6.5	.144	.49	6.05
58	11	159	81	223	107	286	6.1	4.1	.145	.51	5.52
58	12	124	91	227	120	289	5.7	4.5	.140	.58	5.90
59	11	160	75	174	107	302	5.3	4.0	.164	.52	5.95
59	12	297	68	119	106	297	5.5	4.2	.139	.80	5.10
510	11	283	50	115	100	276	4.6	3.5	.124	.54	6.04
510	12	180	90	121	130	718	7.1	6.2	.130	.72	5.40
511	11	95	76	109	91	456	7.8	6.8	.134	.23	4.98
511	12	198	75	158	105	439	6.1	5.2	.145	.92	5.10
512	11	283	59	136	74	659	8.4	6.5	.118	.24	5.96
512	12	327	89	173	107	710	7.4	7.4	.140	.70	5.75
513	11	202	66	92	63	418	7.4	6.2	.114	.42	5.60
513	12	247	67	121	76	853	8.0	8.9	.144	.36	6.10
514	11	204	83	190	60	403	9.0	7.8	.156	.19	6.22
514	12	240	74	162	79	656	8.3	7.4	.127	.57	6.05
515	11	196	79	201	65	409	8.6	7.5	.144	.19	6.30
515	12	257	77	197	73	601	8.5	8.1	.142	.43	6.40
516	11	204	66	158	74	773	6.1	4.7	.095	.83	5.50
516	12	457	145	208	79	913	6.6	6.6	.203	.50	5.85
517	13	245	80	95	90	700	7.1	5.9	.115	.32	5.45
518	13	260	105	160	80	500	8.2	7.1	.130	.20	5.90
519	13	205	75	50	75	550	5.5	5.1	.085	.70	4.70
520	13	55	30	20	35	125	1.1	.7	.025	2.10	4.45
60	13	60	40	30	80	150	.5	.9	.040	1.60	4.60
61	11	46	33	32	60	111	.8	1.0	.048	1.71	5.21

61	12	76	50	38	107	115	.9	1.9	.046	1.80	5.30
62	11	120	54	35	53	616	6.3	6.0	.106	.27	5.10
62	12	159	76	45	57	832	8.6	8.3	.113	.53	5.00
63	11	132	82	113	82	348	6.7	5.6	.121	.46	4.54
63	12	360	120	320	128	360	10.8	7.6	.144	.80	5.55
64	11	129	64	107	74	249	4.7	3.8	.132	.39	4.70
64	12	158	64	86	75	263	5.6	5.2	.135	.75	5.00
65	11	138	53	73	61	380	4.5	3.9	.087	.41	5.00
65	12	186	81	154	97	363	6.8	6.8	.117	.77	5.15
66	11	334	106	637	106	376	6.1	5.8	.131	.45	5.63
66	12	224	62	84	84	734	4.9	6.2	.099	.48	4.90
67	11	81	57	69	95	205	4.7	2.7	.172	.39	5.66
67	12	78	55	98	118	118	3.5	2.7	.149	.82	5.30
68	11	64	48	36	63	198	3.0	2.1	.123	.33	5.45
68	12	76	53	53	84	190	4.5	3.0	.144	.42	5.00
69	11	46	39	32	55	87	2.8	1.3	.104	.51	5.24
610	11	81	53	48	76	436	3.7	2.4	.133	.56	5.71
610	12	75	53	45	75	281	4.5	3.3	.131	.64	5.30
611	11	252	75	291	79	517	6.3	5.6	.148	.57	5.48
611	12	270	85	213	92	579	7.5	7.7	.158	.70	5.00
612	11	174	60	73	72	467	6.2	4.8	.106	.42	5.20
612	12	229	81	406	74	406	5.7	5.5	.118	.81	5.60
613	11	216	87	241	86	460	6.9	5.7	.147	.44	6.29
613	12	222	68	171	86	581	7.8	7.5	.140	.51	5.50
614	11	275	76	387	74	742	7.6	6.3	.126	.34	6.30
614	12	307	99	241	95	876	9.1	8.3	.153	.51	5.60
615	11	299	69	113	68	757	6.2	6.5	.120	.44	4.88
615	12	286	73	46	66	656	9.2	9.2	.135	.54	5.90
616	11	239	84	177	65	608	8.5	7.0	.147	.36	5.20
616	12	281	88	168	76	882	9.6	8.8	.136	.78	4.85
617	11	219	80	196	66	713	7.8	6.5	.138	.34	6.19
618	11	239	83	159	55	697	8.1	7.1	.139	.50	6.18
619	11	463	114	208	80	1325	7.6	7.5	.176	.20	6.35
619	13	275	80	135	85	900	6.7	6.1	.120	.36	5.95
620	13	255	95	235	105	800	7.2	6.8	.140	.40	5.75
70	13	110	60	50	165	195	.6	1.4	.050	2.00	5.10
71	13	50	35	20	75	120	.4	.9	.040	1.67	4.10
72	13	90	55	50	125	190	.7	1.6	.070	2.17	4.55
73	13	55	40	45	70	195	.6	.9	.040	1.45	4.20
74	13	210	85	165	90	370	4.5	5.0	.100	.52	5.00
75	13	340	95	165	130	975	5.5	6.2	.110	.45	5.80
76	13	225	100	165	100	250	4.5	4.1	.120	.37	5.35
77	13	85	75	95	120	125	3.4	2.4	.105	.45	4.85
78	13	50	45	35	60	60	1.9	1.0	.075	.70	4.75
79	13	50	50	35	100	60	2.6	1.5	.140	.28	4.70
710	13	145	80	140	105	375	4.5	4.1	.120	.55	5.15
711	13	170	85	95	115	500	5.0	3.9	.130	.50	5.35
712	13	190	80	55	90	750	6.5	5.5	.140	.38	5.30

713	13	260	90	170	90	825	7.0	6.6	.125	.38	4.55
714	13	315	95	275	95	775	6.2	6.8	.130	.32	4.85
715	13	320	80	60	95	1200	7.8	7.9	.125	.28	5.20
716	13	250	80	30	110	900	7.0	6.2	.115	.60	5.65
717	13	300	75	55	75	1325	8.1	7.0	.130	.28	6.20
718	13	220	70	20	65	950	8.3	7.5	.105	.20	4.75
719	13	320	75	65	95	1200	6.4	6.4	.120	.28	5.90
720	13	40	35	20	40	30	1.2	.6	.020	2.38	5.05

SOILS -120 MESH

SITE NO.	IDENT.	NI	CO	CU	ZN	CR	CA	MG	MN	K
		PPM	PPM	PPM	PPM	PPM	PCENT.	PCENT.	PCENT.	PCENT.
10	23	265	110	280	95	680	8.40	8.50	.120	1.45
11	22	69	34	62	73	236	1.72	1.93	.060	3.34
12	21	40	20	30	40	150	1.50	1.30	.060	.89
13	22	190	72	224	112	456	5.28	5.06	.122	1.60
14	22	278	81	307	125	436	4.36	4.36	.141	.87
15	22	142	53	32	85	502	5.35	4.86	.115	1.42
16	22	122	61	92	107	260	4.40	3.35	.159	1.42
17	22	219	66	66	80	658	8.38	7.98	.140	.84
18	22	221	55	17	77	1190	7.01	7.23	.123	.81
19	22	171	49	55	70	700	4.86	5.06	.086	1.01
110	22	157	51	35	67	648	5.34	4.91	.102	1.14
111	22	180	51	16	63	982	6.14	5.93	.106	.78
112	22	146	44	54	63	595	4.22	4.03	.077	.99
113	22	313	60	66	69	1201	7.64	7.30	.109	.62
114	22	255	60	291	76	958	6.78	6.58	.107	.76
115	22	225	51	79	71	747	5.58	5.25	.101	1.05
116	22	201	56	47	90	666	4.14	3.96	.076	2.12
117	22	175	55	26	80	748	4.38	4.02	.088	2.15
118	23	265	70	20	75	1000	7.70	7.50	.100	1.45
119	23	800	185	110	110	2000	6.50	9.00	.280	1.12
120	23	275	60	40	80	900	7.40	7.50	.105	1.32
20	23	30	25	20	55	110	.65	1.00	.035	3.28
21	22	198	62	278	110	366	4.39	3.66	.088	1.02
22	22	156	62	128	89	355	6.04	4.79	.107	1.21
23	22	404	78	191	130	830	4.33	5.78	.105	.94
24	22	254	66	85	108	674	4.81	5.78	.127	.92
25	22	149	50	57	80	514	5.91	5.14	.137	1.45
27	21	155	70	155	120	450	4.30	4.10	.135	.60
28	21	550	105	1800	110	425	5.20	5.60	.190	.23
29	22	242	64	24	80	1107	7.27	6.75	.121	.87
210	22	233	64	33	102	1019	6.19	5.82	.113	1.24
211	22	171	50	14	78	837	5.70	5.16	.099	1.10
212	22	217	61	45	96	801	4.45	4.27	.082	1.50
213	22	208	54	13	71	1246	6.62	6.32	.104	1.26
214	22	271	78	7	89	1388	6.87	6.76	.142	.99
215	22	250	66	16	83	975	7.41	6.83	.129	1.09
216	22	125	46	64	79	339	2.71	2.32	.061	2.03
217	22	357	82	76	117	728	4.00	4.36	.120	1.97
218	23	235	70	40	85	800	6.50	5.30	.100	1.92
219	23	255	75	30	85	1200	9.50	7.75	.135	1.32
220	23	200	55	30	65	740	7.40	6.00	.080	2.02
30	23	60	40	70	80	275	1.10	1.65	.045	2.72

31	21	45	30	50	70	130	.50	.80	.050	1.20
32	22	109	56	139	85	248	3.76	2.63	.079	2.11
33	21	115	50	180	90	280	2.90	2.50	.090	.56
34	22	208	69	135	118	430	4.34	3.81	.120	1.08
35	22	107	59	87	97	194	4.57	3.11	.120	1.11
36	22	143	63	61	121	321	5.53	4.46	.160	1.25
37	22	126	58	26	93	316	6.51	4.46	.130	1.08
38	22	156	63	85	88	356	4.19	3.90	.140	1.37
39	21	185	70	110	95	550	5.05	4.70	.170	.52
310	21	185	60	120	95	625	6.00	6.10	.130	.45
311	22	293	88	317	95	454	7.80	7.57	.160	.95
312	22	280	67	49	104	757	4.90	5.35	.098	1.78
313	22	163	48	54	84	453	3.52	3.17	.080	1.90
314	22	212	54	25	79	832	4.78	4.99	.100	1.87
315	22	108	36	34	63	360	2.97	2.48	.063	2.16
316	22	138	48	58	82	380	3.27	2.59	.069	2.72
317	23	140	55	100	95	600	3.00	3.20	.055	2.62
318	23	220	75	95	85	820	6.20	5.50	.095	1.82
319	23	800	175	100	120	1200	3.40	6.25	.200	1.72
320	23	300	70	45	85	1000	3.20	3.50	.055	2.80
40	23	45	30	35	65	140	.50	.85	.035	2.80
41	22	55	32	25	78	119	.92	.92	.060	4.49
42	21	45	30	50	60	160	1.15	1.20	.060	1.15
43	22	58	31	38	75	146	1.11	1.33	.062	3.72
44	22	134	53	113	78	336	4.83	4.20	.117	1.22
45	22	305	82	223	106	422	5.08	6.26	.187	1.02
46	22	90	58	57	121	137	5.28	4.30	.152	1.25
47	22	162	63	87	105	396	5.45	5.05	.133	1.17
48	22	150	59	99	99	386	4.93	4.14	.114	.87
49	22	222	70	181	109	639	5.77	5.77	.140	.99
410	22	207	73	145	104	621	5.80	6.21	.137	1.45
411	22	60	36	32	57	127	3.37	1.98	.099	2.34
412	21	270	85	255	100	700	5.50	6.20	.160	.50
413	22	239	92	104	87	809	9.24	8.09	.181	.62
414	22	301	89	51	117	637	5.31	5.66	.131	1.20
415	22	230	69	17	77	960	8.86	6.53	.134	1.08
416	22	126	48	48	70	414	3.33	3.33	.077	3.00
417	23	205	70	25	75	500	5.80	5.50	.085	1.92
418	23	185	75	30	85	555	6.20	5.25	.095	1.45
419	23	400	110	20	90	2000	7.90	7.00	.130	1.32
420	23	220	55	55	75	1000	2.50	2.90	.050	2.80
50	23	90	50	75	135	185	.55	1.10	.050	2.80
51	22	135	48	205	66	294	3.48	2.71	.112	1.63
52	22	295	85	351	117	384	4.44	4.04	.129	1.70
53	22	146	63	47	77	534	6.48	5.67	.129	1.05
54	22	209	82	232	105	372	6.08	5.32	.125	1.29
55	22	190	65	131	95	428	5.74	5.35	.127	1.15
56	22	142	62	135	98	291	4.73	4.00	.105	1.13

57	22	476	138	884	118	590	7.07	6.88	.176	1.02
58	22	184	88	326	147	245	5.51	4.49	.142	1.02
59	22	133	64	148	123	243	5.50	4.17	.162	1.52
510	22	289	85	170	135	574	6.93	6.73	.190	1.27
511	22	204	75	226	132	351	6.03	4.90	.185	1.89
512	22	313	84	222	116	582	7.10	6.55	.146	1.27
513	22	279	76	160	92	758	8.18	8.78	.160	.64
514	22	269	80	254	99	546	7.77	7.58	.133	1.17
515	22	241	77	246	88	544	8.34	7.46	.136	.79
516	22	434	133	1045	95	804	5.03	4.82	.209	1.13
517	23	245	75	105	80	600	7.10	6.00	.105	1.32
518	23	260	110	150	75	400	6.90	5.80	.115	1.00
519	23	160	65	70	60	380	4.00	3.90	.060	2.20
520	23	45	30	20	30	100	.85	.70	.015	3.80
60	23	45	40	30	70	140	.50	.70	.025	3.12
61	22	84	46	51	124	135	1.26	1.26	.055	4.21
62	22	156	67	47	70	821	7.42	7.04	.113	.78
63	22	366	104	408	136	306	5.75	6.38	.128	1.23
64	22	145	61	100	92	267	5.57	4.46	.139	1.28
65	22	147	69	171	98	293	5.62	4.89	.102	1.37
66	22	241	58	104	98	682	4.51	5.41	.116	.84
67	22	87	45	102	117	94	3.14	1.97	.141	1.10
68	22	73	46	46	87	154	4.25	2.32	.142	.81
69	21	35	35	30	60	70	2.30	1.05	.105	.52
610	22	78	41	46	83	221	3.58	2.30	.129	1.15
611	22	277	76	234	108	511	6.50	6.28	.151	1.21
612	22	261	66	525	91	436	5.45	5.45	.132	1.56
613	22	266	76	242	95	546	7.60	7.13	.147	1.24
614	22	307	83	293	95	699	6.99	6.99	.140	.95
615	22	304	68	63	77	638	7.73	8.21	.140	1.01
616	22	274	78	220	88	709	7.82	7.83	.132	.93
617	21	250	85	265	95	900	6.30	6.35	.150	.43
618	21	270	80	200	80	675	6.05	6.50	.130	.26
619	23	300	95	165	85	800	7.00	6.50	.110	1.52
620	23	245	95	225	90	550	6.20	5.75	.120	1.52
70	23	90	55	55	160	150	.75	1.40	.055	3.05
71	23	40	30	50	70	120	.50	1.00	.035	2.95
72	23	80	45	65	110	140	.80	1.60	.045	3.27
73	23	40	30	25	60	135	.55	.95	.030	2.88
74	23	180	65	145	85	500	4.25	5.00	.080	1.75
75	23	350	90	190	125	700	5.80	6.00	.090	1.52
76	23	170	85	195	90	200	4.30	4.00	.100	1.45
77	23	50	55	65	90	120	2.75	2.30	.080	1.32
78	23	35	45	65	80	50	1.95	1.30	.080	2.12
79	23	35	45	60	100	55	2.40	1.65	.150	1.22
710	23	125	70	170	100	350	4.25	4.20	.105	1.72
711	23	130	80	100	105	425	4.40	4.10	.110	1.52
712	23	160	70	50	85	650	7.00	5.75	.120	1.52

713	23	245	85	155	85	725	7.50	7.00	.115	1.60
714	23	260	85	275	85	600	6.80	7.00	.120	1.45
715	23	310	80	60	85	950	8.20	8.00	.115	1.32
716	23	185	60	85	85	625	6.50	5.50	.090	1.95
717	23	300	75	80	80	1000	8.20	7.00	.120	1.45
718	23	195	65	40	65	800	8.60	7.50	.095	1.22
719	23	280	70	70	90	850	7.10	6.75	.105	1.45
720	23	25	30	15	35	40	.85	.75	.015	4.02

SOILS -400 MESH

SITE NO.	IDENT.	NI PPM	CO PPM	CU PPM	ZN PPM	CR PPM	CA PCENT.	MG PCENT.	MN PCENT.	K PCENT.
10	33	255	105	395	95	725	7.50	9.00	.140	.45
11	32	65	25	55	70	240	1.45	1.50	.060	1.19
13	32	175	70	205	120	500	4.70	4.00	.115	.76
14	32	295	85	285	150	550	4.40	4.40	.135	.45
15	32	145	55	45	90	655	5.60	4.40	.110	.64
16	32	130	63	88	120	365	4.90	3.96	.145	.58
17	32	215	66	65	85	820	8.10	8.00	.135	.40
18	32	230	56	15	85	1400	7.10	7.50	.125	.33
19	32	165	45	55	80	835	4.50	4.30	.090	.49
110	32	165	53	73	100	800	5.20	4.45	.100	.52
111	32	175	50	15	70	1050	6.00	5.50	.100	.40
112	32	140	45	50	70	740	4.00	3.65	.075	.49
113	32	310	61	68	75	1210	7.25	7.70	.105	.30
114	32	260	61	28	80	1050	6.20	7.00	.110	.33
115	32	210	50	83	85	850	4.80	5.10	.105	.52
116	32	205	56	50	100	800	3.50	3.55	.100	.79
117	32	185	58	65	95	850	3.70	3.60	.080	.88
118	33	270	70	60	75	950	6.00	7.40	.110	.50
119	33	556	145	225	115	1500	5.70	8.20	.240	.30
120	33	260	60	55	75	800	5.10	6.40	.110	.30
20	33	35	35	20	55	85	.60	.95	.060	1.60
21	32	205	70	250	130	500	4.25	3.50	.090	.42
22	32	160	68	120	95	450	5.80	4.50	.105	.45
23	32	430	86	210	150	920	4.00	5.10	.095	.36
24	32	255	70	90	125	825	4.50	5.65	.105	.33
25	32	160	48	60	95	610	6.05	5.25	.130	.61
27	31	145	65	140	105	440	3.95	3.75	.125	.60
28	31	575	115	2400	110	375	4.70	5.20	.170	.31
29	32	245	71	28	90	1115	7.10	7.20	.150	.40
210	32	235	70	37	120	1025	5.50	5.40	.120	.58
211	32	165	54	20	90	870	4.90	4.50	.115	.52
212	32	225	67	48	110	850	3.75	3.70	.100	.73
213	32	195	55	20	130	1100	5.15	5.35	.100	.58
214	32	270	90	10	100	1210	6.00	6.95	.155	.49
215	32	260	75	20	90	1040	7.00	7.00	.135	.52
216	32	120	45	53	80	490	3.00	2.50	.075	.88
217	32	340	83	93	125	825	3.90	4.30	.120	.82
218	33	215	65	60	85	650	4.50	4.25	.110	.80
219	33	280	80	110	90	1050	7.40	6.70	.145	.40
220	33	215	70	70	75	850	6.50	6.15	.105	.80
30	33	58	40	60	70	190	.95	1.30	.060	1.32
31	31	45	30	50	75	120	.50	.90	.055	1.09

32	32	120	55	143	90	360	3.50	2.90	.085	.76
33	31	100	45	160	85	250	2.40	2.15	.090	.68
34	32	220	76	153	135	580	4.25	4.05	.120	.52
35	32	130	65	88	105	380	5.00	3.65	.125	.52
36	32	145	66	68	130	440	5.50	4.15	.150	.58
37	32	135	59	33	100	415	6.55	4.55	.135	.52
38	32	135	56	78	85	455	4.15	3.40	.120	.70
39	31	125	50	95	65	390	3.80	3.70	.120	.39
310	31	200	70	160	95	675	5.95	6.05	.140	.43
311	32	295	98	310	95	630	7.75	8.50	.155	.40
312	32	295	74	65	115	850	4.20	4.60	.100	.79
313	32	160	52	63	90	600	3.10	3.00	.085	1.15
314	32	205	51	35	85	810	3.80	4.00	.110	.85
315	32	105	38	40	65	450	2.50	2.00	.065	.97
316	32	140	47	68	85	500	2.75	2.40	.080	1.22
317	33	130	50	75	70	525	2.05	2.20	.065	1.00
318	33	223	70	60	85	825	5.00	5.00	.110	.72
319	33	590	130	75	100	825	2.90	6.00	.200	.60
320	33	300	75	65	90	800	2.75	3.35	.080	1.40
40	33	48	40	35	70	105	.30	.75	.055	1.40
41	32	60	29	28	75	120	.85	1.00	.075	1.82
42	31	45	25	65	55	160	1.90	1.95	.065	.18
44	32	130	50	103	75	420	4.25	3.30	.115	.70
45	32	315	91	225	115	550	4.50	5.55	.200	.45
46	32	100	56	73	115	150	4.25	3.45	.125	.45
47	32	175	64	85	100	490	4.80	4.30	.125	.49
48	32	160	60	100	100	480	4.40	3.50	.100	.36
49	32	225	71	175	115	790	5.50	5.50	.100	.49
410	32	200	80	138	105	190	5.75	6.30	.140	.58
411	32	60	44	35	60	150	3.40	2.05	.125	.85
412	31	80	25	105	40	230	1.85	2.00	.070	.21
413	32	245	110	108	95	1000	9.00	8.50	.185	.25
414	32	290	95	60	120	800	4.75	5.75	.135	.52
415	32	230	75	20	85	1000	7.50	7.75	.130	.45
416	32	115	50	50	70	500	3.35	2.90	.075	1.09
417	33	203	70	20	80	470	5.00	5.20	.100	.80
418	33	190	75	40	90	575	5.50	5.30	.110	.45
419	33	308	105	60	100	1650	6.50	6.90	.150	.45
420	33	238	65	40	85	900	2.75	2.75	.075	1.32
50	33	83	55	75	125	145	.55	1.00	.065	1.40
51	32	110	35	165	60	290	2.20	1.80	.100	.61
52	32	350	95	380	155	550	4.00	4.50	.130	.68
53	32	130	55	55	70	550	5.40	5.20	.125	.40
54	32	225	76	245	110	450	5.50	5.20	.125	.49
55	32	195	75	130	95	570	5.40	5.40	.130	.52
56	32	165	65	137	100	400	4.30	4.10	.115	.52
57	32	650	172	1150	135	800	7.25	8.20	.200	.58
58	32	235	101	360	160	400	5.50	5.15	.150	.45

59	32	175	68	175	145	360	5.70	4.55	.185	.76
510	32	285	101	210	145	710	5.70	7.20	.210	.52
511	32	195	84	235	155	480	6.00	5.70	.205	.88
512	32	320	92	265	130	760	7.40	8.50	.175	.55
513	32	290	90	165	90	910	8.25	9.50	.170	.30
514	32	300	84	285	100	740	8.15	8.25	.155	.45
515	32	270	88	250	100	740	8.50	9.40	.160	.33
516	32	460	140	950	100	900	4.90	5.05	.195	.52
517	33	240	75	115	85	625	6.00	5.95	.120	.50
518	33	250	110	195	80	400	5.30	6.60	.130	.30
519	33	175	75	195	75	450	4.75	4.40	.090	1.20
520	33	53	45	40	50	120	1.15	.80	.040	2.68
60	33	48	40	40	65	120	.40	.65	.050	1.52
61	32	90	51	60	120	150	1.50	1.70	.070	1.70
62	32	170	66	50	70	900	6.80	7.80	.125	.33
63	32	450	123	430	165	450	5.45	6.65	.140	.52
64	32	165	65	90	95	320	5.45	3.80	.135	.49
65	32	190	64	175	100	380	5.00	4.50	.100	.58
66	32	245	64	103	100	830	4.60	5.30	.110	.43
67	32	105	55	103	145	150	3.40	2.45	.160	.49
68	32	100	50	43	95	190	4.50	2.60	.155	.40
69	31	40	30	45	55	90	.80	.95	.065	1.07
610	32	85	45	42	85	260	3.60	2.20	.135	.40
611	32	285	75	20	110	680	7.15	7.90	.160	.55
612	32	265	70	435	100	550	6.00	6.25	.145	.49
613	32	275	80	230	100	690	7.75	7.80	.155	.64
614	32	330	93	300	105	870	6.50	7.50	.145	.52
615	32	330	80	75	85	860	8.00	9.00	.150	.61
616	32	295	84	185	100	860	7.80	8.15	.140	.37
617	31	260	90	395	105	750	6.05	6.40	.145	.43
618	31	270	80	205	80	650	5.60	6.00	.130	.29
619	33	320	100	195	100	850	6.40	7.00	.150	.50
620	30	240	85	265	105	600	5.90	5.85	.150	.55
70	33	94	55	80	155	130	.70	1.25	.065	1.48
71	33	45	30	60	65	125	.70	1.00	.055	1.40
72	33	73	40	135	115	105	.55	1.35	.060	1.75
73	33	35	30	65	55	95	.35	.75	.055	1.32
74	33	17	55	175	85	500	3.85	4.50	.100	.68
75	33	388	95	295	140	725	4.25	5.80	.120	.50
76	33	150	65	240	85	170	3.75	3.30	.120	.40
77	33	55	45	110	80	75	2.80	1.90	.090	.30
78	33	25	35	145	60	50	1.40	.85	.080	.50
79	33	30	45	60	100	45	2.65	1.35	.175	.30
710	33	108	55	240	90	280	3.45	3.30	.120	.50
711	33	128	70	130	110	375	3.50	3.45	.125	.45
712	33	130	55	75	75	500	5.00	4.40	.125	.45
713	33	225	75	175	80	650	5.90	5.90	.120	.45
714	33	260	80	410	100	575	5.50	6.75	.140	.40

715	33	253	65	140	70	825	6.00	6.50	.120	.30
716	33	193	65	70	95	650	5.00	5.00	.110	.72
717	33	275	75	235	90	1000	6.50	6.25	.130	.35
718	33	208	70	40	65	825	7.70	8.00	.120	.35
719	33	293	75	90	100	925	6.05	7.10	.140	.45
720	33	50	45	75	45	90	1.40	1.05	.040	2.58

SOILS HCL EXTRACT

SITE NO.	IDENT.	NI PPM	CU PPM	ZN PPM	CR PPM	CA PERCENT	MG PERCENT	K PERCENT
10	43	50.0	12.3	10.5	24.0	1310	5200	134
11	42	5.0	9.0	5.0	28.0	100	500	103
12	41	2.0	4.8	3.8	8.0	625	250	78
13	42	16.8	60.0	10.8	37.0	2100	1880	145
14	42	16.5	37.5	11.8	27.0	675	2000	90
15	42	4.5	6.0	4.3	32.0	125	400	90
16	42	5.3	12.0	10.5	10.0	375	600	125
17	42	3.9	27.5	7.0	30.0	1125	600	103
18	42	8.5	3.3	3.5	32.0	800	725	78
19	42	8.0	7.8	6.8	44.0	300	675	90
110	42	4.8	5.0	5.3	39.0	400	500	62
111	42	4.8	2.5	3.8	42.0	550	463	43
112	2	3.2	7.5	4.8	46.0	100	375	78
113	42	3.3	11.5	4.0	33.0	625	325	50
114	42	9.2	55.0	4.3	62.0	400	875	50
115	42	8.5	14.5	5.3	44.0	275	550	78
116	42	7.8	6.0	8.0	50.0	150	438	70
117	42	5.5	3.5	6.3	57.0	250	400	78
118	43	20.3	4.3	8.0	52.5	413	2000	88
119	43	375.0	12.5	40.0	12.5	1600	9999	350
120	43	18.0	9.3	6.8	48.5	1050	2500	92
20	43	2.3	2.3	4.8	7.3	88	400	186
21	42	11.0	50.0	12.5	30.0	500	325	138
22	42	5.8	26.5	3.8	19.0	275	450	103
23	42	15.8	23.0	8.8	58.0	200	1310	55
24	42	14.0	16.2	7.8	48.0	150	1125	82
25	42	5.0	6.5	3.8	34.0	1250	825	98
27	41	9.0	21.5	11.0	28.0	225	1680	188
28	41	125.0	875.0	16.3	30.0	1660	6880	132
29	42	7.8	2.5	5.3	46.0	750	925	55
210	42	14.2	5.0	11.0	54.0	575	775	98
211	42	6.2	1.8	3.3	40.0	725	538	35
212	42	11.0	5.8	9.8	57.0	163	375	78
213	42	8.2	1.2	4.5	37.0	163	350	50
214	42	21.8	1.0	10.8	52.0	175	1000	70
215	42	6.8	2.0	4.5	40.0	275	450	50
216	42	4.3	7.3	6.8	34.0	125	413	103
217	42	32.5	13.5	16.8	57.0	450	2050	110
218	43	17.3	8.5	9.8	42.0	950	1175	115
219	43	16.5	7.0	11.5	41.0	1175	1420	164
220	43	11.5	6.3	6.0	27.5	638	663	101
30	43	3.3	7.3	6.3	16.3	50	350	106

31	41	3.5	4.5	6.8	10.0	100	450	78
32	42	12.0	23.3	11.8	39.0	213	1840	118
33	41	5.5	26.5	10.0	27.0	200	325	305
34	42	8.5	14.8	8.5	33.0	175	800	125
35	42	3.0	8.0	4.3	13.0	200	488	132
36	42	3.5	7.5	6.5	19.0	550	575	125
37	42	1.5	2.5	2.5	16.0	125	250	55
38	42	11.3	19.0	10.0	34.0	1075	2430	320
39	41	15.8	24.0	8.0	28.0	350	1500	110
310	41	11.8	21.3	7.8	26.0	525	1510	78
311	42	50.0	14.3	13.8	28.0	2300	6000	62
312	42	11.5	6.5	9.3	40.0	463	863	55
313	42	6.5	8.8	7.5	37.0	375	750	42
314	42	10.5	3.5	6.8	41.0	175	750	90
315	42	2.5	4.0	4.5	30.0	100	300	55
316	42	5.5	9.5	8.5	32.0	450	675	70
317	43	3.5	6.3	6.0	37.0	113	350	88
318	43	10.3	6.8	7.5	57.5	563	950	101
319	43	22.5	17.5	27.5	75.0	1200	7500	245
320	43	16.5	7.0	9.8	67.5	338	925	92
40	43	3.0	3.5	5.3	9.8	63	400	128
41	42	2.8	3.0	7.3	11.0	75	500	103
42	41	2.8	3.8	5.0	14.0	138	250	90
43	41	3.0	2.8	7.0	13.0	100	400	82
44	42	6.3	15.0	3.5	23.0	75	600	62
45	42	40.0	70.0	16.3	34.0	2100	5500	80
46	42	1.8	5.0	4.5	5.0	275	350	78
47	42	5.3	12.0	6.8	22.0	138	1025	98
48	42	8.8	15.0	9.0	26.0	175	1125	98
49	42	8.5	26.2	7.5	46.0	250	700	78
410	42	8.5	26.1	9.0	52.0	500	2430	103
411	42	4.0	5.5	13.3	10.0	850	2100	222
412	41	36.1	90.0	13.8	55.0	2070	5630	125
413	42	20.5	31.5	13.8	48.0	1280	2160	125
414	42	35.0	10.0	19.0	50.0	825	3500	150
415	42	7.0	3.0	4.0	40.0	413	875	50
416	42	5.0	8.0	6.5	35.0	150	625	62
417	43	7.3	2.3	3.5	15.8	325	625	43
418	43	5.5	3.5	11.8	32.2	975	563	115
419	43	31.3	3.5	16.5	100.0	625	1680	106
420	43	14.5	4.0	10.5	95.0	525	513	101
50	43	6.0	8.5	25.0	16.3	63	388	84
51	42	20.0	3.4	5.0	18.0	325	550	98
52	42	29.0	65.0	21.3	49.0	400	4000	98
53	42	4.3	5.5	3.3	13.0	525	363	42
54	2	6.3	24.5	6.3	21.0	375	500	42
55	42	9.3	21.0	5.8	31.0	175	500	78
56	42	6.0	21.5	7.8	16.0	175	775	300

57	42	60.0	210.0	13.8	30.0	2630	4000	118
58	42	23.0	80.0	23.3	20.0	2750	4380	208
59	42	16.0	32.5	22.5	16.0	3500	4500	335
510	42	32.5	36.8	16.8	38.0	4000	4500	110
511	42	25.0	67.5	19.8	21.0	3630	4880	300
512	42	33.8	80.0	13.0	33.0	3500	4250	110
513	42	36.2	62.5	10.3	33.0	3250	9250	110
514	42	36.0	100.0	10.3	25.0	4380	7000	103
515	42	26.2	122.5	9.0	30.0	2880	6000	62
516	42	65.0	337.0	15.0	68.0	1630	2000	125
517	43	12.8	28.7	6.5	26.2	750	1320	122
518	43	20.8	46.2	10.3	21.8	850	2160	70
519	43	8.8	13.5	7.5	23.5	175	1013	134
520	43	2.0	2.3	3.0	15.3	63	125	72
60	43	1.7	3.5	4.3	7.8	63	150	96
61	42	4.5	6.5	17.3	15.0	100	563	138
62	42	9.5	12.0	3.8	30.0	213	500	55
63	42	41.2	107.5	19.5	21.0	625	338	50
64	42	3.8	12.0	5.3	14.0	188	950	82
65	42	3.5	21.5	3.0	20.0	100	325	103
66	42	12.6	17.5	5.8	45.0	100	875	132
67	42	4.5	14.0	20.3	3.0	375	1600	138
68	42	2.5	6.8	12.0	8.0	125	1150	98
69	41	1.8	4.3	12.0	3.0	125	1000	118
610	42	4.3	11.3	16.0	13.0	275	1025	125
611	42	35.3	97.5	11.8	34.0	1500	5250	70
612	42	32.0	170.0	9.8	37.0	3000	4880	270
613	42	31.2	95.0	8.5	26.0	3750	4750	200
614	42	28.5	97.5	9.8	49.0	1250	3380	138
615	42	36.2	22.5	8.8	65.0	3130	6750	90
616	42	29.2	85.0	10.5	40.0	1000	4880	145
617	43	34.2	117.5	14.3	45.0	2750	5000	118
618	43	33.7	80.0	11.0	48.0	1125	3380	82
619	41	175.0	132.5	23.8	76.0	1630	5880	118
620	43	32.5	82.5	15.0	36.5	2380	5000	400
70	43	4.8	6.5	37.5	10.8	38	250	94
71	43	3.0	4.3	7.0	10.0	113	288	155
72	43	6.0	10.8	11.5	8.8	113	813	142
73	43	2.5	3.5	4.3	12.3	119	325	58
74	43	18.8	37.5	10.5	32.5	688	1940	128
75	43	25.0	50.0	16.5	40.0	338	1450	76
76	43	7.3	47.5	6.8	8.3	163	925	76
77	43	3.0	12.0	12.8	5.8	238	1250	125
78	43	3.3	5.8	21.5	2.8	525	2440	220
79	43	3.3	6.0	25.0	2.8	875	3006	212
710	43	3.8	25.0	5.3	17.0	625	975	128
711	43	6.0	18.5	12.8	18.3	463	600	58
712	43	5.8	8.3	4.8	30.0	675	600	128

713	43	20.5	50.0	6.8	46.0	175	1480	92
714	43	11.5	50.0	7.3	38.7	238	1690	67
715	43	25.0	19.3	11.8	52.5	563	1400	156
716	43	7.3	5.5	8.5	33.5	725	575	106
717	43	50.0	27.5	9.3	90.0	188	500	61
718	43	3.5	2.8	2.3	23.5	175	488	61
719	43	26.3	22.3	13.5	50.0	1000	3100	298
720	43	1.0	2.3	3.8	2.0	100	188	88

PHYSICAL VARIABLES

SITE NO.	SLOPE DEGREES	ALTITUDE FEET	TREE HT. FEET	TREE DIAM INCHES	SAMP. HT. FRACTION
10	55	2420	10	1.5	.50
11	45	2370	12	.9	1.00
13	55	2250	15	1.5	.50
14	35	2230	30	3.0	.40
15	25	2200	6	.4	1.00
16	35	2130	6	.7	.50
17	35	2120	12	1.4	.40
18	30	2170	8	1.4	.90
19	20	2190	15	1.1	.70
110	20	2220	25	2.1	.30
111	10	2220	20	2.0	.30
112	10	2210	50	6.0	.20
113	30	2170	70	22.0	.40
114	40	2120	15	1.4	.30
115	25	2080	40	2.8	.20
116	35	2030	50	5.0	.20
117	35	1980	30	3.8	.30
118	30	1910	20	3.0	.80
119	30	1860	30	3.0	.30
120	20	1770	100	24.0	.20
20	30	2430	25	2.0	.80
21	50	2390	6	.5	.80
22	35	2370	15	1.3	.50
23	30	2350	20	2.3	.50
24	35	2300	6	1.0	.30
25	30	2250	20	2.1	.40
29	40	2070	6	.5	.50
210	40	2100	40	4.0	.30
211	45	2130	30	1.8	.30
212	25	2130	16	1.1	.90
213	25	2120	15	1.4	.40
214	30	2080	10	1.0	.50
215	40	2050	10	.9	1.00
216	10	2020	40	3.0	.20
217	40	2080	40	6.0	.20
218	25	2030	50	4.0	.20
219	25	1870	25	2.5	.60
220	30	1800	20	2.0	.90
30	10	2540	8	1.0	1.00
32	35	2450	7	.8	.70
34	35	2330	60	7.0	.10
35	30	2280	10	1.0	1.00

36	40	2220	10	.9	1.00
37	35	2150	60	12.0	.10
38	40	2090	40	2.5	.40
311	40	1980	9	1.0	.60
312	40	2020	120	30.0	.10
313	25	2030	30	2.4	.20
314	30	2020	20	1.5	.40
315	20	2010	8	.8	.90
316	20	1970	20	.8	.80
317	30	1930	25	1.5	.20
318	45	1880	20	2.0	.50
319	55	1830	8	1.5	.60
320	45	1770	40	12.0	.30
40	30	2550	35	2.0	.70
41	25	2500	25	2.0	.40
43	25	2400	4	.5	1.00
44	35	2340	20	1.4	.30
45	25	2300	20	1.4	1.00
46	35	2250	8	.9	.50
47	40	2190	25	1.5	.30
48	40	2140	12	.9	.50
49	35	2100	8	.6	.80
410	40	2030	10	.9	.50
411	45	1960	10	.9	.90
413	45	1870	10	.8	.80
414	30	1900	80	12.0	.10
415	40	1870	100	17.0	.10
416	25	1910	10	.9	1.00
417	30	1870	40	3.0	.20
418	35	1810	40	6.0	.20
419	35	1750	20	1.0	.90
420	40	1710	8	1.0	.80
50	10	2530	30	4.0	1.00
51	10	2520	18	1.1	.90
52	20	2480	14	1.4	.60
53	25	2460	20	2.5	.40
55	30	2360	15	2.0	.70
56	35	2290	15	1.3	.50
57	40	2260	18	1.4	.40
58	50	2170	90	20.0	.10
59	35	2110	6	.6	1.00
510	45	2020	9	1.0	1.00
511	35	1950	80	12.0	.10
512	45	1930	12	1.3	.50
513	55	1840	20	2.3	.10
514	40	1750	15	1.1	.70
515	45	1750	18	2.0	.30
516	45	1770	12	1.0	1.00

518	30	1740	6	.5	1.00
519	35	1700	20	2.0	1.00
520	35	1640	25	2.0	.80
60	30	2510	20	2.5	1.00
61	25	2480	10	1.0	1.00
62	20	2450	40	3.5	.20
63	40	2420	7	.8	1.00
64	30	2410	4	.4	1.00
65	20	2380	10	1.0	.60
66	30	2340	3	.3	1.00
67	40	2300	5	.8	.60
68	40	2220	15	1.0	.90
610	40	2110	20	1.5	.40
611	40	2000	6	.5	.80
612	40	1930	15	1.4	.80
613	45	1860	8	.6	1.00
614	60	1790	8	.6	1.00
615	45	1730	40	3.5	.40
616	50	1680	10	1.0	.10
620	30	1570	45	4.0	.10
70	30	2420	30	1.5	.70
71	40	2380	12	1.5	.50
72	30	2350	12	1.5	.50
73	15	2330	35	7.0	.20
74	30	2320	25	2.5	.80
75	25	2310	20	3.5	.50
76	30	2290	80	14.0	.10
77	45	2250	6	1.0	1.00
78	40	2200	20	3.0	.50
79	10	2160	30	3.0	.80
710	40	2110	20	2.0	.40
711	40	2050	18	1.5	.90
712	50	1980	20	1.5	.40
713	30	1940	38	4.0	1.00
714	30	1910	20	1.5	.50
715	50	1850	20	1.0	.80
716	20	1800	20	1.0	.90
717	20	1770	20	1.5	.30
718	30	1730	30	2.0	.20
719	50	1710	20	1.5	.50
720	10	1680	20	1.5	.30

APPENDIX VII.

Publications arising from this thesis.

Timperley, M.H., Brooks, R.R., and Peterson, P.J., 1970, Prospecting for copper and nickel in New Zealand by statistical analysis of biogeochemical data: Econ. Geol., v.65, p.505-510.

Timperley, M.H., Brooks, R.R., and Peterson, P.J., 1970, The significance of essential and non-essential trace elements in plants in relation to biogeochemical prospecting: J. appl. Ecol., v.7, p.429-439.

Timperley, M.H., Brooks, R.R., and Peterson, P.J., 1971, The improved prediction of geochemical soil anomalies by multiple regression analysis of biogeochemical data: Submitted to; Proc. Australas. Inst. Min. Metall.

Timperley, M.H., Brooks, R.R., and Peterson, P.J., 1971, Trend analysis as an aid to the comparison and interpretation of biogeochemical and geochemical data: Submitted to; Econ. Geol.