ASPECTS OF RADIOBIOGEOCHEMISTRY

A thesis

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To my parents,

Dr. and Mrs. V.I.E. Whitehead

with gratitude.

" This I know;

God made man simple,

Man's complex problems

Are of his own devising."

(Ecc. 7.29, Jerusalem Bible)

- SOLOMON (961 - 922 B.C.)

ABSTRACT

Section I: A routine assay for uranium was instituted, and a fluorimeter suitable for the assay designed and constructed. A new method of fusion-pellet manufacture is described. The optimum conditions for the assay were determined. The calibration curve is linear up to about three micrograms of uranium; the lowest limit of detection is about twenty nanograms (about 0.10 microgram/g of sample). Routine alpha and beta counting of samples was developed.

Section II: The forms of naturally occurring data distributions are discussed, and customary methods of examining these noted, together with their defects. A versatile computer programme was developed to determine the form of natural distributions, and to calculate correlation coefficients and their significances.

Section III: An orientation survey of a known mineralised area in the Buller Gorge of New Zealand showed that C.australis, N.fusca, Q.acutifolia, and W.racemosa are suitable for biogeochemical prospecting for uranium. Analysis figures were more nearly log-normally distributed than normally distributed, and multiple distributions were often present. Alpha counts of plant material also proved suitable as indicators of the amount of uranium in the soil, as did the amount of iron in the leaves.

Section IV: Aquatic bryophytes from streams draining mineralised areas were analysed and the results found to be indicative of the presence or otherwise of uranium in the various catchment areas. Even better was the use of specially prepared peat, allowed to soak in the stream water. The accumulation factor for uranium, from stream water, was about ten thousand.

Section V: The gamma-ray spectra of plants and soils were carefully characterised by solvent extraction, and ion-exchange techniques. Plants were found to absorb radium and uranium and lead, but not thorium. B.procerum, and M.berteroana, however absorbed both thorium and actinium. Calculation showed that most of the alpha particles emitted by the samples studied were from ^{238}U.

Section VI: Extraction and characterisation of uranium complexes in C.australis leaves showed the presence of a protein-uranium complex, and an RNA-uranium complex. The latter is at least partially an artefact of the extraction technique, and examination of fresh material showed that more than half the uranium was bound to cell wall proteins. No other types of compound besides protein and nucleic acids possessed measurable binding capacity for uranium.

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GENERAL INTRODUCTION

The continued improvement in the living standards of the developed countries is vitally dependent on a large supply of uranium.

The above apparently radical statement, is in reality only common sense, since living standards are very closely linked to energy availability, and at present nuclear power seems to be the only practical means of producing the large amount of electricity which will be needed in the future. Most other conventional means of generating electricity are either already utilised to the full, or are uneconomic. It should also be remembered that the cost of electricity generated by nuclear fission is still falling, as new and more efficient reactor designs are introduced.

Two other factors render nuclear reactors attractive and important. The pollution of the environment is minimal compared with fossil fuels, and the cheapness of the process raises the possibility of other applications such as desalination, which is much needed in many parts of the world.

The economic aspects of nuclear power are important even for New Zealand, with its apparently abundant hydroelectric resources. In fact, the rising costs of hydroelectric schemes due partly to increasingly-remote locations of the power stations, will render nuclear power essential in New Zealand within two decades, and for this reason, it would be of advantage to this country's economy if local and substantial deposits of uranium ore could be found.

In 1956, two prospectors, Cassin and Jacobsen, found a radioactive trachyte dyke in the Buller Gorge (South Island of New Zealand), and a flurry of prospecting activity followed. With mutual co-operation between Lime and

Marble Ltd of New Zealand, and the United Kingdom Atomic Energy Authority, deposits of uranium ore were soon discovered on the north and south banks of the Buller Gorge. The work was suspended about 1959-60 because of a world drop in the price of uranium, but the initial ground scintillometry had already been extended by Wodzicki (1959a and 1959b) to include sampling of stream waters, and radioactive boulders in stream beds.

Towards the end of the 1960's the world price of uranium firmed sufficiently to render further prospecting attractive, and Cohen et.al.(1967, 1969) sampled soils and stream sediments to give further indications of favourable areas for more detailed study.

The above listed methods do not exhaust the types of prospecting methods which may be used in a region. Other possible methods may be derived from the concept of Fortescue and Hornbrook (1967). These authors considered a three dimensional section of rock, soil and vegetation, as shown in fig. 0.1. Assuming that the mineralisation was buried deeply it could theoretically be detected by examination of the rock if outcrops existed or, rather more indirectly, by analysing other more remote parts of the prism, such as the soil, vegetation, or even the air (to detect in this latter case, radon, a daughter product of uranium.) In general these more remote sections of the prism or cylinder, have been unduly neglected.

Analysis of vegetation, to gain information about the underlying material, is well suited to a heavily bushed area, and is known as biogeochemical prospecting. The technique has been reviewed by Malyuga (1964) and Cannon (1960b) and has been used fairly widely in New Zealand, with studies on nickel, chromium, cobalt and copper (Lyon et al. 1968; Timperley et al.

1970), molybdenum and copper (Brooks and Lyon, 1966; Lyon and Brooks, 1969) and zinc, copper and lead (Nicolas and Brooks, 1969). The work had, however, never included an investigation on uranium deposits.

For a well-rounded survey of the region under study, it seemed important to attempt at least a preliminary study of the possibilities of biogeochemical prospecting for uranium under the conditions found in New Zealand. Results already found overseas could not be directly applied since the vegetation types were quite different.

Uranium is radioactive, and has potentially associated with it a large number of daughter products, such as various isotopes of thorium, radium, polonium and lead. These can be detected by various means, and it is interesting to determine how these elements are absorbed by plants, and whether any of them are useful in the context of prospecting, as tracers, or 'pathfinders' for uranium. This also applied to other trace elements involved in the metabolism of the plant.

Uranium is not only radioactive, but poisonous as well, and its toxic effects on metabolism are usually more significant than the radiation it produces. How can a plant survive the absorption of such an element? Research was obviously needed here. Such avenues of basic research were considered essential to a continuing expansion of knowledge concerning the factors that enable plants to be used in biogeochemical prospecting.

The aims of the thesis were thus specifically:

(i) To develop a routine, but sensitive assay for uranium on the basis

of previous work, to enable the low levels of uranium in plants and soils to be readily determined.

(ii) To collect a number of plants and soils from a mineralised area, to find which, if any, of the species were suitable for biogeochemical prospecting by analysis for uranium, and to compare the use of chemical methods and alpha and beta counting as indicators (direct or otherwise) of uranium mineralisation.

(iii) To analyse the plants and their corresponding soils for a number of other elements connected with trace element metabolism, and see whether pathfinders for uranium existed.

(iv) To develop simple statistical techniques and a computer programme for handling all the above data.

(v) To examine any other parts of the 'prospecting prism' which appeared useful.

(vi) To develop a technique of simultaneously estimating the amounts of all gamma-emitting isotopes in the samples by the use of gamma-ray spectrometry in the low-energy region, and from this to determine which isotopes were producing the bulk of the alpha rays in the sample.

(vii) To find the nature of the chemical form of uranium in plant tissue.

SECTION I

ANALYTICAL

METHODS

ANALYTICAL METHODS.

It has been known for many years, that the fusion of sodium fluoride with uranium produces a white solid showing intense golden-yellow fluorescence under ultraviolet light. (Nichols and Slattery 1926). This is the basis of a sensitive quantitative assay for uranium, and this section describes the conditions chosen, and the type of fluorimeter constructed.

A. Preparation of plants and soils for analysis.

1. Sampling

Approximately 20 g of leaves was collected from each plant, together with about 30 g of wood selected from 20 mm diameter twigs which had been cut into small sections with secateurs. Most of the specimens were collected during three field trips, each three months apart, in February, May, and August 1968. Seasonal effects would not be expected in the specimens sampled, which are evergreens, but care was taken to sample a number of leaves of different ages from different parts of each plant, and to prepare a composite from them. There was no apparent difference in relative accumulation of uranium or radioactivity for samples taken during these three field trips. Check resampling of certain specimens gave essentially the same results after a period of two years.

A total of about 250 g of soil was also taken from the B horizon at various points around the base of each plant. Samples were packed in plastic bags and transported to the laboratory.

2. Washing of plant specimens

Cannon (1953) mentions that washing plant samples from a mineralised area appears to remove a portion of the uranium, the part removed being

presumably contamination in the form of windblown dust.

Froelich and Kleinhampl (1960) appear to disagree with this statement when they write

'The washing in water of plant samples obtained from areas of mining activity where contamination by uraniferous dust has occurred, generally does not alter their composition.'

These differences of opinion may be due to washing time, or species of plants sampled, but we considered it wisest in this work to wash plant samples thoroughly but briefly in tap water. The problem of windblown dust is not so serious in New Zealand, where there is dense vegetation cover and high rainfall.

3. Ashing procedure

Malyuga (1964) states that no significant uranium is lost from plant material by dry-ashing at 500^{0}C or even higher. This point deserves comment, for some elements, e.g. polonium, are completely volatilised at these temperatures, and others are partially volatilised. (Cleary and Hamilton, 1968, Pijck et. al., 1960)

Washed plant tissues were dried at 110^{0}C for several hours, weighed, and ashed in 100ml Pyrex beakers using a muffle furnace. Eighteen hours at 500^{0}C was generally sufficient to remove carbonaceous matter. The weights of ash were then recorded.

Soils were air-dried, sieved through a 40-mesh nylon sieve, ground in an agate mortar, and after ignition at 500^{0}C were further ground to pass a 100 mesh sieve.

4. Dissolution of plant ash and soils

Plant ash was dissolved in 3 M nitric acid, as it has been shown by Geiger (1959) that this treatment solubilises almost all uranium.

Soils, however, required digestion with ten times their weight of 1:1 hydrochloric-hydrofluoric acid mixture. The mixture was evaporated to dryness in 15ml polypropylene beakers partially immersed in a water bath, and redissolved in 15ml of 3 M nitric acid.

The suitability of the method was checked by analysis in triplicate of CAAS syenite (Webber, 1965) for which uranium values of 2,890 ppm were obtained (recommended range 2,230 - 3,000 ppm). It was concluded that this method of dissolution was adequate.

B. Development of a fluorimetric method of uranium analysis.

It is sometimes possible, without preliminary purification, to fuse a sample directly with sodium fluoride and measure the fluorescence produced. Grimaldi et al. (1952) have described one such method in use in the laboratories of the United States Geological Survey. However many elements normally present in plants and soils act as quite efficient quenchers of the fluorescence, unless a very small sample is used. Leonova, quoted by Nemodruk and Voronitskaya (1962), has shown that if less than 10 ppm uranium are present, preliminary purification is essential. Since concentrations of uranium are not known a priori it seems a major saving of time to institute a routine method of extracting uranium from impurities.

The mass of the fusion pellet may also be important. Samsoni (1967) has shown that the fluorescence may, up to a limiting value, depend on the ratio of pellet mass to mass of uranium. It is necessary to have some way of standardising pellet weights, if large numbers of analyses are performed. Price et al. (1953) manufactured pellets by pressure against a hard flat surface using a topless syringe, but our work shows other methods are possible, and probably less time consuming.

1. Solvent extraction.

Various methods of purification of uranium used in the past, have included solvent extraction, column chromatography (Ostle, 1954; Grodzinskii and Golubkova 1963) and an ingenious method utilising the strong affinity of uranium for protein (Glover, 1953; Neuman et al., 1948). Column chromatography is impractical for large numbers of samples, and the purification using protein is slow, involving a forty-five minute coagulation step. Solvent extraction is therefore the method of choice.

Solvent systems used have included, ethyl ether (Grodzinskii and Golubkova, 1963), tri-n-octylamine (Moore, 1958; Medvedeva et al., 1964; Tserkovnitskaya and Bykhovtseva, 1964) a mixture of tri-n-butyl phosphate and methyl-isobutyl ketone (Vera-Palomino et al., 1964) a solution of tri-n-butyl phosphate in kerosene or other non-polar solvent (Geiger, 1959; Nemodruk and Vorotnitskaya, 1962; Sato 1965), and ethyl acetate (Grimaldi et al., 1954; Guest and Zimmerman, 1955; Ohashi, 1961). In initial trials the common ethyl acetate method did not yield consistent results, and tri-n-butyl phosphate in kerosene was adopted as a solvent.

Following the procedure of Sato (1965), uranium was extracted from solutions of plant ash or soil obtained according to the procedure shown in part I.A.4. by shaking with a 40% (v/v) solution of tri-n-butyl phosphate in kerosene. One ml of sample solution was shaken with 1.2ml of tri-n-butyl phosphate solution for five minutes followed by either centrifugation, or standing. Sato (1965) has shown that five minutes shaking is necessary for equilibrium. One ml of the non-aqueous phase was pipetted into a fresh tube, and 1.2ml of 10% ammonium carbonate added to back-extract the uranium. After a similar period of shaking and settling, the top, kerosene layer, was removed by suction, and 1.0ml of the layer was pipetted into a gold dish, capacity 1.0ml. The solution was then evaporated to dryness in the vestibule of the muffle furnace.

To test how thoroughly quenchers were removed by this system, a sample of plant ash containing no detectable uranium, was extracted as described above and added to a known amount of uranium. No significant quenching occurred.

2. Pellet manufacture

Since the melting point of pure sodium fluoride is very high, it is usual to add sodium and potssium carbonates as a flux. The mixture most commonly used, and employed in this work (Grimaldi et. al. 1952), was $NaF:Na_2CO_3:K_2CO_3$ 9:45.5:45.5, though the fluorescence is slightly less than with pure sodium fluoride.

Two hundred grams of the dry ingredients, in the above proportions, were mixed with 85 ml of distilled water, and blended twice at high speed in a Waring blendor for fifteen seconds. The resulting paste was poured into a series of moulds, comprising 9 mm diameter holes drilled through a 9 mm thick Teflon plate, and placed on a glass sheet. After drying for 8 hours at 105°C, the pellets were extruded with a suitable sized plunger, and further dried at the same temperature for a day. The mean weight of the pellets thus obtained was 1.0 g. Pellets were sorted into weight ranges 0.05 g apart, and only a single weight range used for a given determination, though, since no noticeable effect could be seen with similar samples and different pellet weights, this precaution may have been unnecessary.

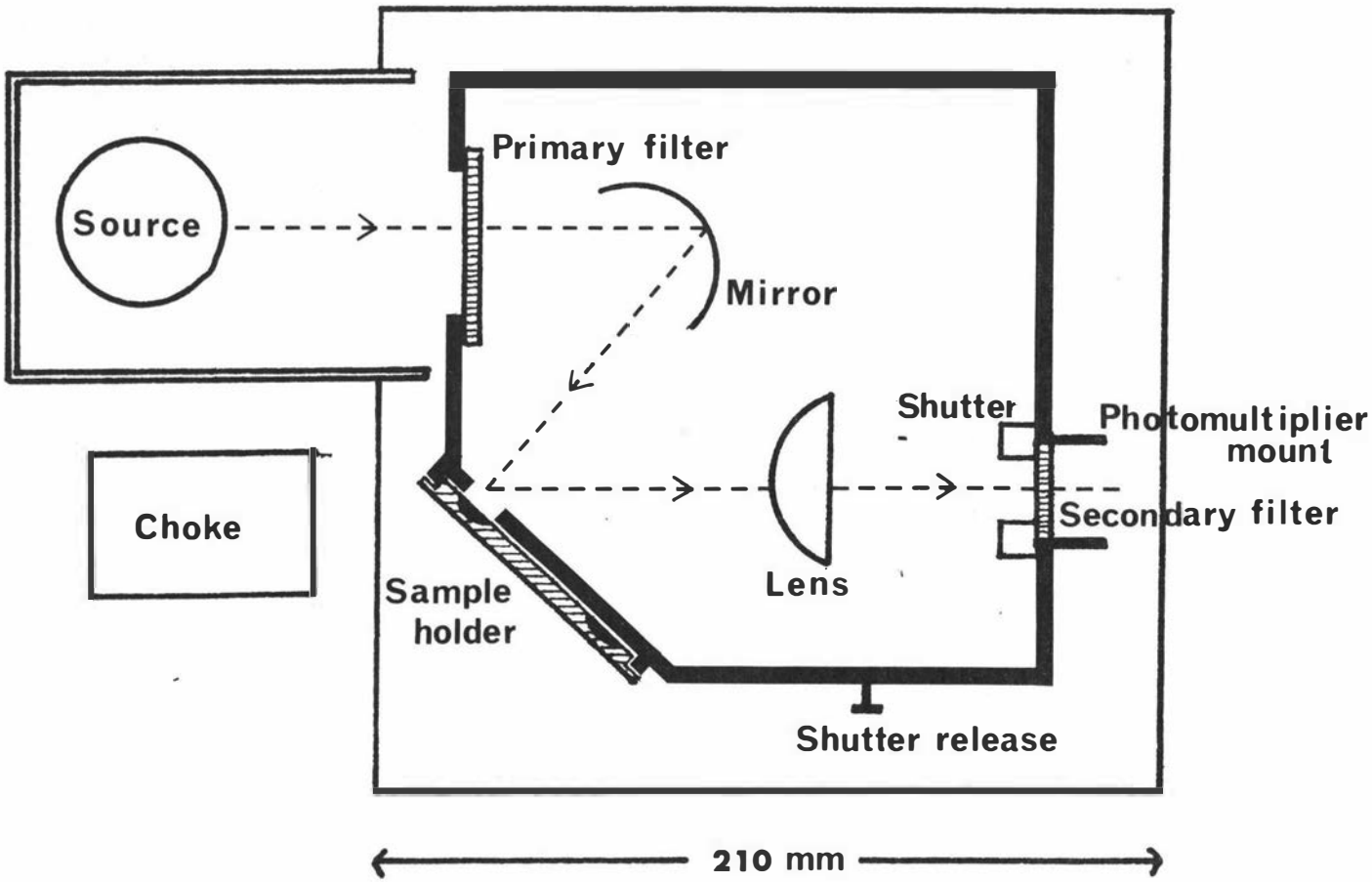
C. Development of a fluorimeter.

Two main types of fluorimeter have been developed for quantitative measurement of solid state fluorescence and involve the principles of reflectance and transmission respectively. In the former case, the fluorescence is measured from the same side of the pellet as the ultraviolet light source, whereas in the latter, it is the fluorescent radiation passing through the pellet which is measured.

Fletcher et. al. (1954) claim that a transmission arrangement gives greater sensitivity because the source may then be very much closer to the pellet, and the incident radiation be much more intense. It is necessary to remove the pellet from its fusion dish however, and in initial trials this often proved impossible. It is also difficult to guarantee constant pellet depth, and accordingly a reflectance-type geometry was chosen (Brooks and Whitehead, 1968). This geometry is illustrated in fig I.1.

1. Light source

The light source was a Phillips 125 w mercury-discharge ultraviolet lamp mounted on a three-pin base. The outer cobalt-glass envelope was removed to increase the light intensity, and the lamp was enclosed in a lamp housing, constructed of 18-gauge galvanised iron sheet. The housing was 110x80 mm, 200 mm high, and surrounded the source on three sides. A small flue was incorporated at the top to allow dissipation of heat. A 125 w choke was incorporated in series with the lamp, and mounted adjacent to it, on a wooden base. The source, after switching on, required ten minutes for stabilisation.


Source
Primary filter
Mirror
Shutter
Photomultiplier mount
Secondary filter
Choke
Lens
Sample holder
Shutter release
210 mm

2. Optical path

The optical system was enclosed within a 160 mm x 160 mm metal housing (18 gauge galvanised iron), 100 mm high and was mounted on another metal base to bring the optical path into lateral alignment with the source. The base was 60 mm high; other dimensions may be found on fig.1. Access to the inside of the optical housing, was by a lid with twelve screws and wing nuts, and light leaks around the lid were eliminated by use of a rubber gasket. All inside surfaces were painted black.

Light from the source entered the box via a 50 mm diameter Chance OX1 glass filter transmitting optimally at 365 nm. The incident light was focussed on to the samples, in a rotating sample holder by means of a 100 mm focal length front-silvered mirror. The fused bead in the holder emitted secondary fluorescent radiation, which was focussed by a 50 mm planoconvex lens on to a 1P21 photomuliplier through filters. During initial trials it was found necessary to place between the mirror and the lens a baffle to eliminate stray light, which was doubling the level of the blank.

The filters were a combination of Wratten 2A and W12 filters (which gave the highest signal to blank ratio) mounted inside a 29 mm o.d. cylinder which served as a mounting for the photomultiplier housing. The photomultiplier was protected by a metal-leaf camera shutter controlled by a cable release with clutch, mounted on the side of the instrument.

3. Sample holder

The rotating sample holder, designed to take nine dishes, containing fused pellets, is illustrated in fig.I.2. Basically it consists of a 90 mm diameter brass disk (thickness 15 mm) with a series of nine hemispherical

cavities drilled at regular intervals around the circumference of the holder. These recesses were machined to fit gold fusion dishes with an outside diameter of 18-20 mm. The centre of each cavity was 27 mm from the centre of the holder. The outside edge of the disk had a flange 4 mm wide and 3 mm deep, making the overall diameter 98 mm. A rubber O-ring fitted over the flange and was cemented to it with an epoxy resin. The fusion dishes were held inside the holder with an 18 gauge brass retaining disk with holes (11mm in diameter) which exactly corresponded with the recesses in the holder. The retaining disk was held in place with a grub screw.

The holder assembly fitted into a 90 mm inside diameter cylinder of length 10 mm, which in turn was soldered to the optical housing. An 8 mm hole was cut in this housing exactly corresponding with the cavities in the sample holder as it was rotated within the cylinder. Rotation was aided by lubrication of the rubber O-ring with silicone grease.

In order to avoid the introduction of extraneous light, the sample holder was pressed against the end of the cylinder by means of a transverse bar fitting over two protruding screws mounted on each side of the assembly. A ball bearing soldered to the bar, made contact at the centre of the sample holder. The correct amount of pressure was maintained by two springs mounted over the screws between the bar and a pair of wing nuts. The whole arrangement is illustrated in fig.I.2.

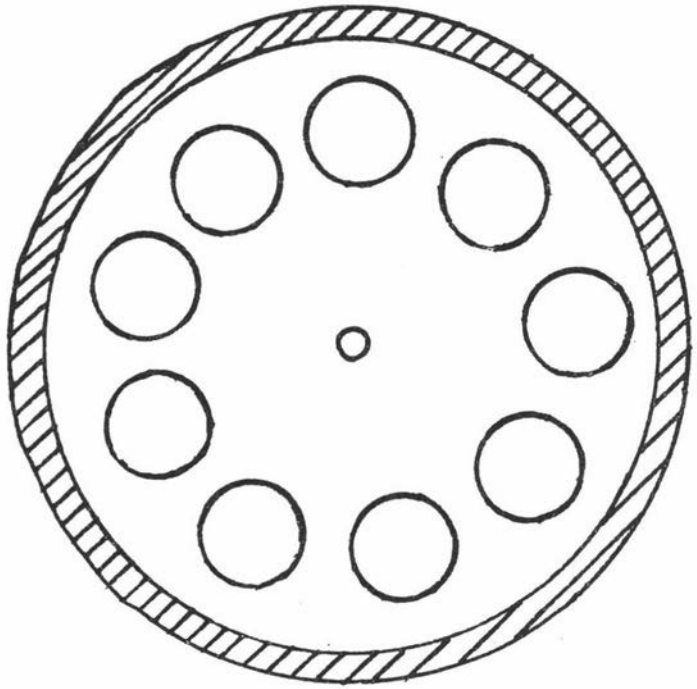
The sample holder was rotated to bring each sample in turn into the optical path of the instrument, by allowing ultraviolet light from the convex mirror to fall upon it. A series of marks on the edge of the holder facilitated correct positioning of each sample, following which adjustment

was made to give a maximum reading.

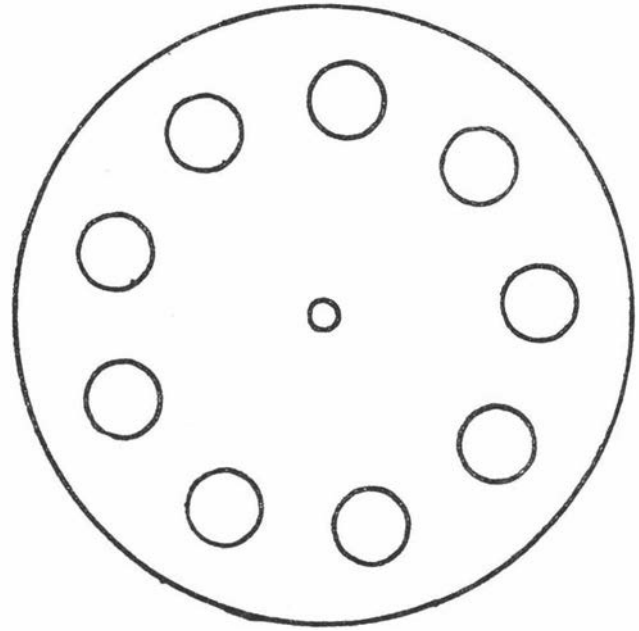
4. Detection system

The photomultiplier mounting was designed to fit exactly the photomultiplier housing from a Techtron AA-3 absorption spectrophotometer. The mounting consisted of a 29 mm o.d. stainless-steel cylinder fitted with two circumferal grooves to take rubber friction rings to ensure a light-proof seal. This system enabled the very stable electronics of the spectrophotometer to be used for amplification, and the fluorescence level to be displayed on the meter of the instrument. It did not prove necessary to use the full amplification available. The AA-3 is tuned to receive only a modulated signal, however since the source in this case was a discharge tube, the mains frequency of 50 Hz was sufficient for proper operation of the detection system.

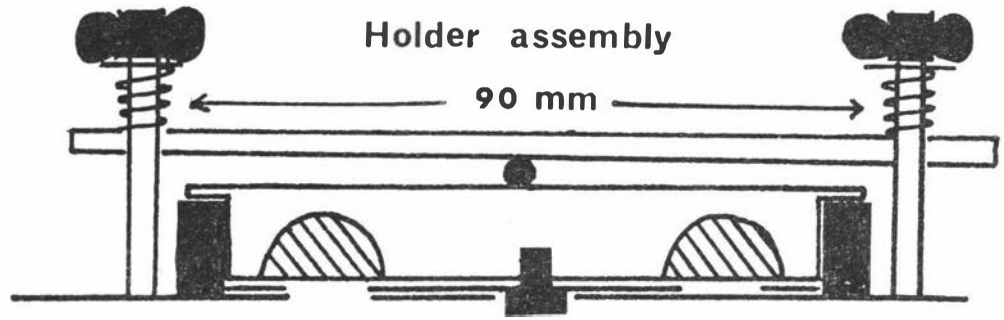
Sample holder



Retaining disk



Holder assembly



D. Application of method.

For carrying out fusions, dishes containing solutions from the procedure in part I.A.2, were laid on top of a special holder. This consisted of a 100 mm x 60 mm x 10 mm stainless-steel plate which was inserted into and withdrawn from the furnace with stainless-steel prongs. Nine lengths of 12.5 mm i.d. silica tubing were mounted on the plate in an upright position. The purpose of these cylinders was to afford a stable mounting for the fusion dishes, and to provide a minimum area of contact between the two surfaces. Also if spillage occurred, this ensured there was no risk of the melt spreading to other dishes.

1. Influence of dish depth

The gold dishes used were moulded from gold sheet by pressure, and tended through many heating-cooling cycles to revert to a flatter shape. Old dishes appeared to give a somewhat higher level for fluorescence than new ones, and this observation was tested by measuring dish depth and, correlating with the average fluorescence produced by a standard amount of uranium in each dish. The correlation coefficient calculated according to the procedure given in part II was -0.94, meaning that there was a very strong inverse relationship between the dish depth and the fluorescence. Calculation of the probability that this result was spurious gave a figure of less than 0.001, so the dishes were calibrated every few months, depending on use, by measuring the fluorescences of known amounts of uranium. When the capacity of the dishes became less than 1.0 ml, they were hollowed out manually with the bottom of a test-tube, and recalibrated.

2. Time of heating

The pellet mixture melts at 605°C (Fletcher, 1954), and all authors recommend that time and temperature of fusion be kept to a minimum. This is because the molten flux tends to attack the gold or platinum dishes used, and both these elements, if dissolved, tend to quench the fluorescence. However if an insufficient time is allowed, the dried uranium solution in the dish will not mix thoroughly with the flux. In order to determine the optimum time for the fusion procedure, standard uranium solutions were assayed using different times for fusion, and the results are shown on fig. I.3. With the furnace set at 650°C the pellets melted in 4 minutes, and maximum fluorescence was reached in approximately 15 minutes. Thereafter a slow decline in fluorescence was noted. For overall ease of operations, the standard time of fusion was set at 20 minutes. The pellets were air-cooled, and the fluorescence was read immediately. Other authors have used similar times (Price et. al., 1953; Samsoni, 1967).

3. Sensitivity

A calibration curve for the fluorimeter is shown on fig. I.4. The line was linear past the section shown up to about 3 mg of uranium, when a lessening in slope was noticed. The lowest calibration point on fig.4. represents 0.05 micrograms of uranium but the lower limit of the method is set by the size of the blanks (i.e. pellets containing no uranium). Blanks were of the order of $(3 \pm 0.5)\%$ when a standard containing 4 mg of uranium gave a full-scale deflection of 100%. What should be considered as the highest sensitivity depends on the acceptable signal to "noise" ratio where "noise" is taken as being the signal from the blank, and this in turn depends on the above-quoted reproducibility of the blank. If a reading of 0.5% above the blank is accepted as being real, this would represent a lower limit of detection of 0.02 mg, or 20 nanograms. This figure was more than adequate and no attempt was

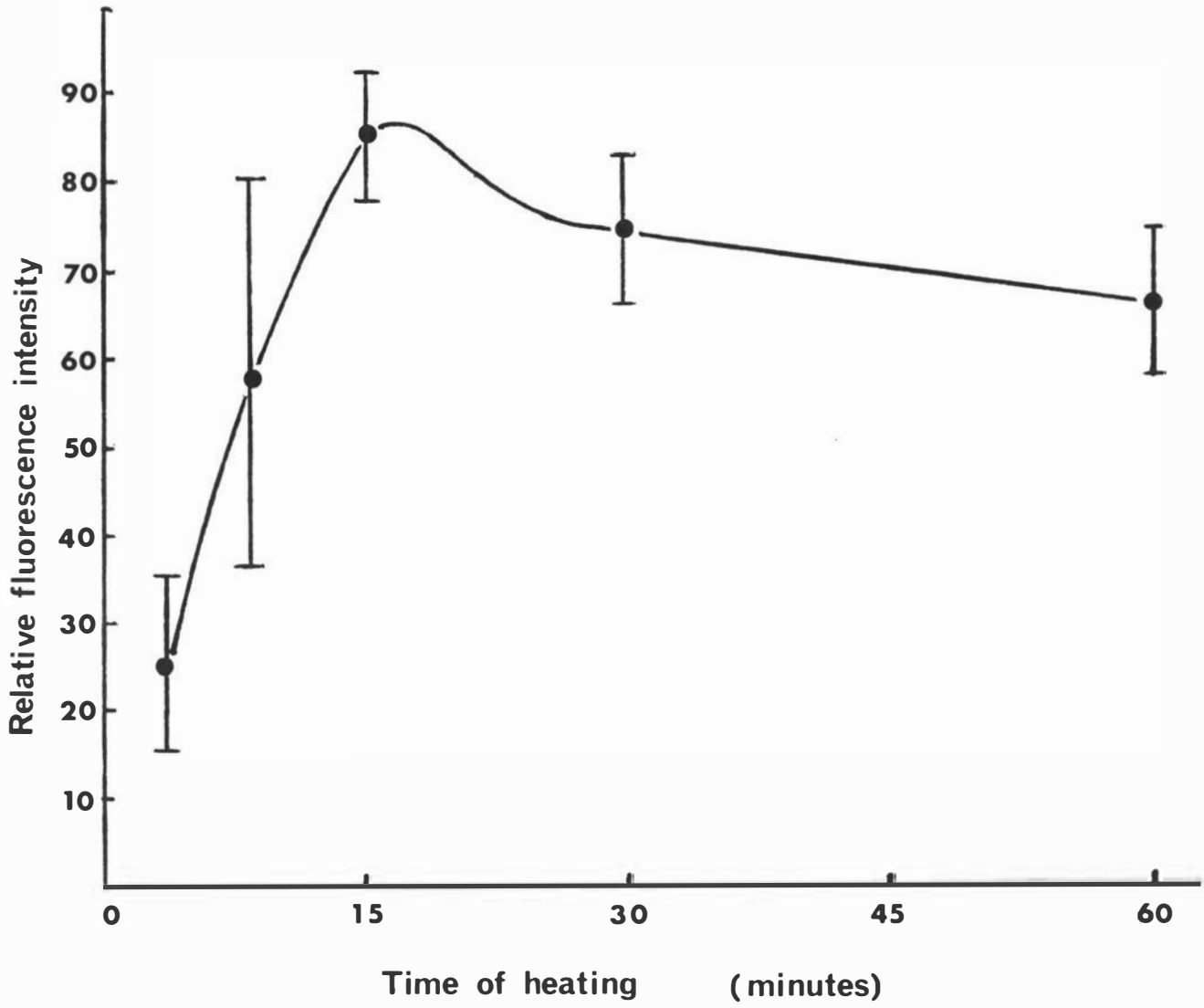


Figure I.3. Variation of uranium fluorescence with heating time

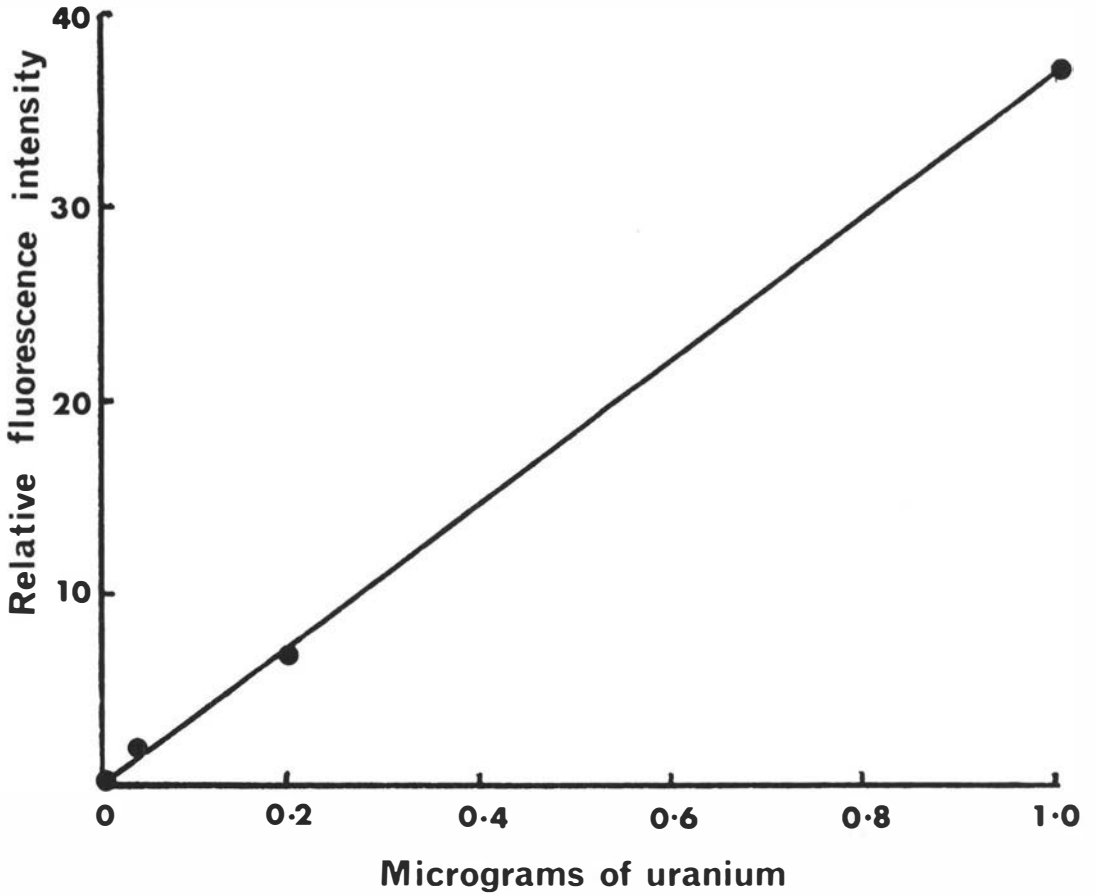


Figure I.4. Calb

made to reduce the blank level further. Other authors quote similar sensitivities, for example, Ohashi and Murakami (1960) and Grodzinskii and Golubkova (1963).

4. Reproducibility

As found by many workers, uranium losses occurred at the solvent extraction step. Overall yields were only 53% (c.f. Geiger, 1959, 76%) but were very consistent. This loss was almost certainly due to the rather low polarity of the nitric acid solutions, but higher concentrations tend to encourage the extraction of thorium, one of the quenchers it was desired to eliminate (Sato, 1965).

The overall reproducibility was found by extracting known amounts of uranium through the solvent extraction procedure, and measuring the fluorescence. For twenty samples the standard deviation as a fraction of the mean was $\pm$ 13%. This is similar to figures reported by others; e.g. 10% (Samsoni, 1967), 15% (Grimaldi *et al.*, 1954), 14% (Geiger, 1959). If great care is taken it may be possible to reduce the error to as low as 7.5% (Nemodruk and Vorotnitskaya, 1962), but the complicated method used by these authors is not suited to routine analysis. As a standard procedure, samples were analysed in duplicate and the mean taken as the true value.

E. Alpha and beta counting

1. Method

(a) plant ash

The alpha and beta activities of samples of soil and plant material were recorded on a Beckman Lowbeta II low-background counter. Counting times of 100 minutes were used.

Plant samples were prepared for counting by dissolving 50 mg of ash in 1.5 ml of 3 M nitric acid, and evaporating 1 ml of this solution to dryness in a stainless-steel planchet.

(b) soil samples

Soil samples were counted by grinding the material (125 mg) with its weight of gum arabic (acacia), and mixing it into a paste with a few drops of water. The paste was dried on the planchet, over a low Bunsen flame. The purpose of this operation was to cement the sample to the planchet in order to avoid the risk of contamination of the instrument. It follows approximately the method of Kunasheva (1939).

2. Evaluation

Alpha and beta particles are relatively easily absorbed by many materials including the above sample materials. This will cause variation in count figures obtained, unless the thickness is exceptionally uniform. One way of avoiding this is to increase the thickness of sample until the count-rate reaches a constant level. This condition is known as counting under conditions of 'infinite' thickness. To determine whether this condition was satisfied for the sample preparation conditions selected, a series of plant and soil sources were prepared with graded amounts of

material, and graphs constructed for sample weight versus count rate, as shown on figs. I.5 and I.6. The conditions for soils are optimum but those for plant ash are not. Unfortunately since uranium and other estimations were usually required, it was normally not possible to spare more than 50 mg of ash for this measurement. How valid the comparative figures for plant ash were, thus depended on the reproducibility of count rate of similar samples.

Two sets of 11 replicates of plant and soil material were counted, and the coefficients of variation were 3.7% and 7.3% respectively, showing that the above conditions were adequate.

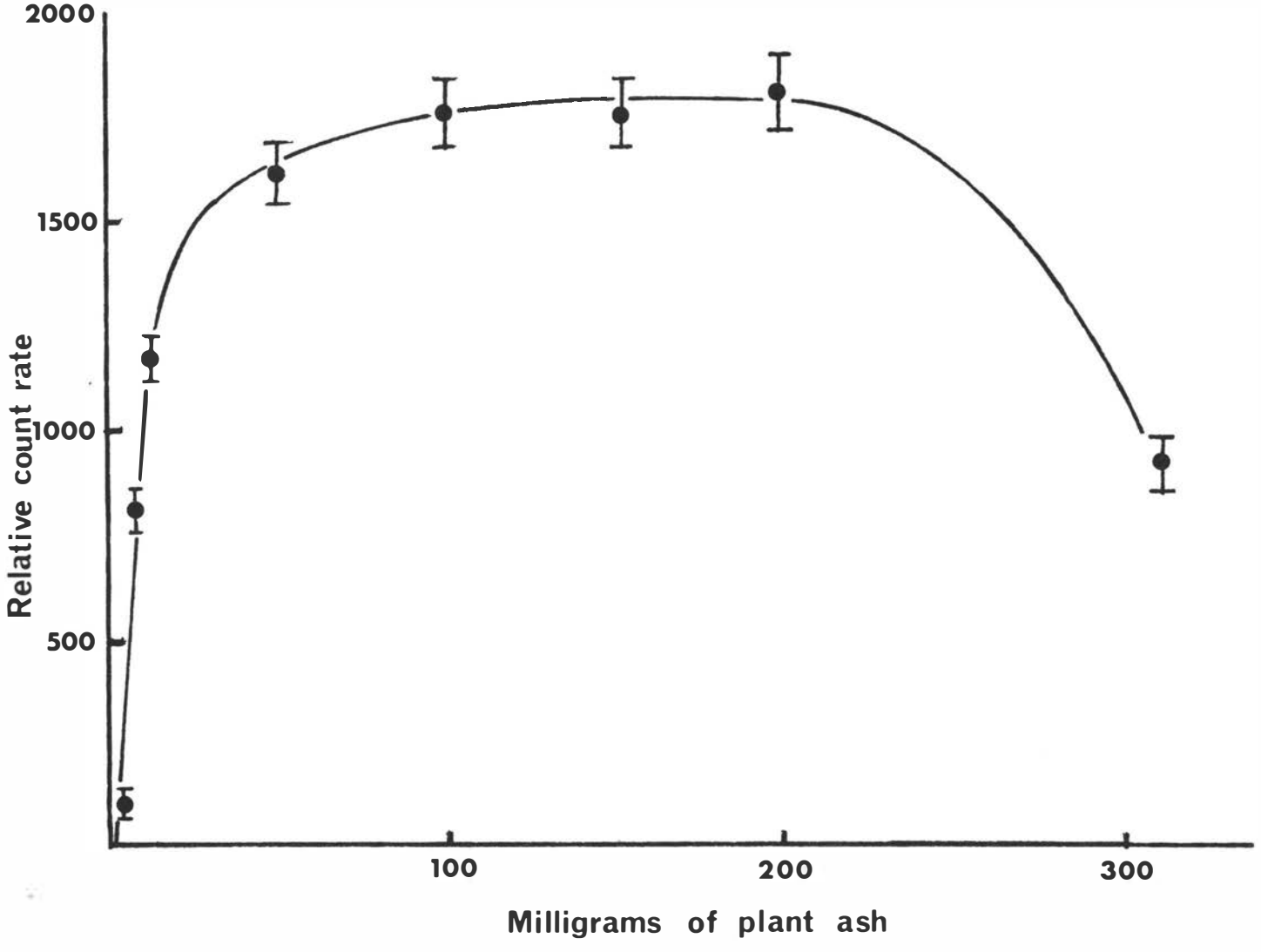
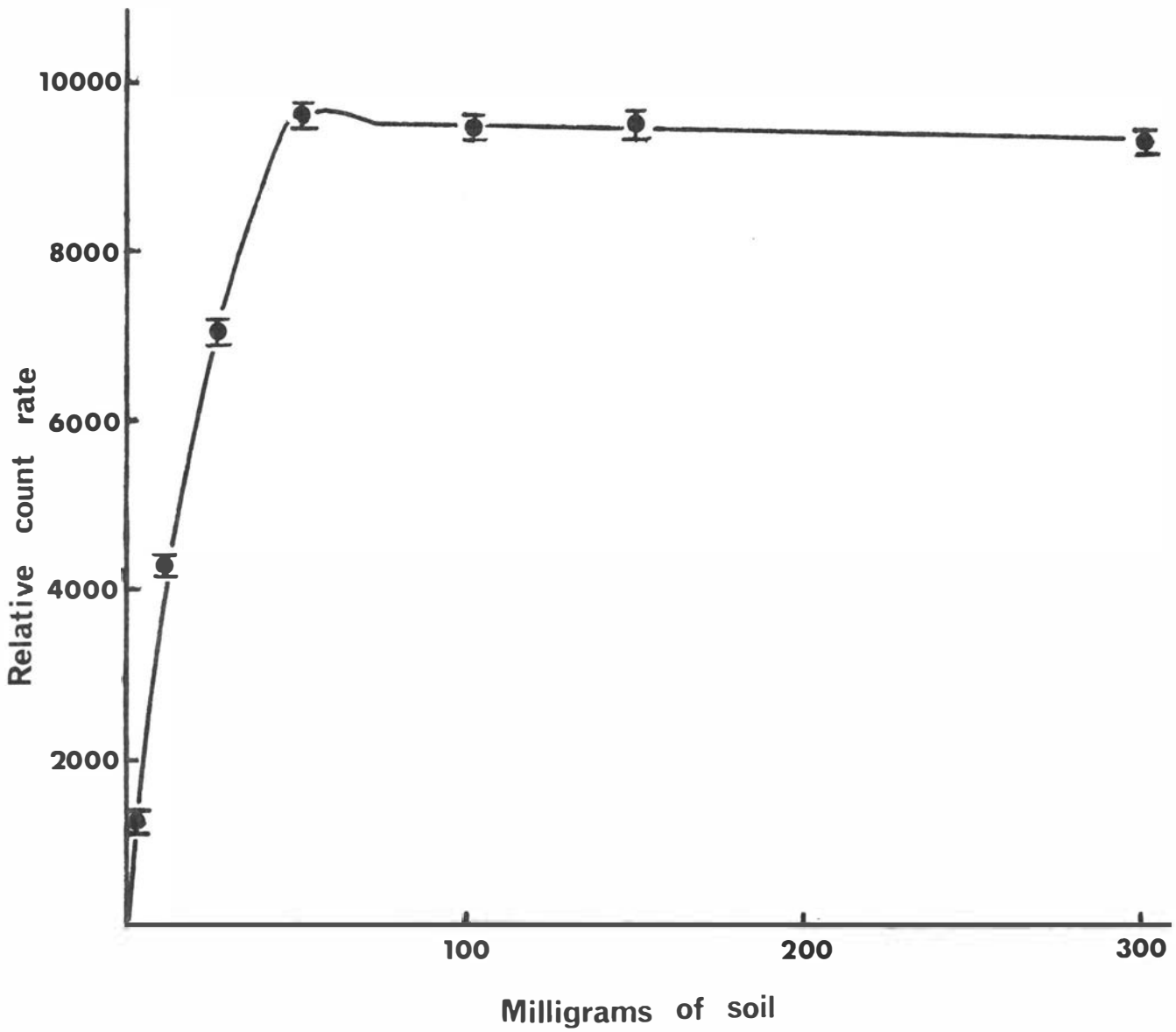


Figure I.5. Variation of alpha count rate with sample thickness; plant samples


10000
8000
6000
4000
2000
Relative count rate
100
200
300
Milligrams of soil

F. Other analytical methods used.

1. Atomic absorption spectrophotometry

Solutions obtained from the dissolution procedures outlined in part I.A.4, were analysed for calcium, copper, magnesium, iron and zinc using a Techtron AA-3 atomic absorption spectrophotometer. Calcium was not determined on soil solutions, because the initial attack with hydrofluoric acid almost certainly precipitates calcium as the fluoride, giving low and variable figures.

2. Flame photometry

Sodium and potassium were determined by flame photometry, using a Gallenkamp instrument.

SECTION II

UTILISATION AND ASSESSMENT OF DATA

This section describes briefly how data are used for prediction in biogeochemistry, examines inherent limitations in any such attempt, and gives a computer programme designed to minimise such limitations.

A. Statistical description of data.

1. Properties of distributions

In general, analytical results obtained in the course of biogeochemical investigations, are random within a certain definite pattern. This is known as a random distribution. It is sometimes possible to construct a mathematical model that accords quite closely with the observed distribution, and if this can be done, then it may be possible to predict quantitatively, the probability of a fresh result falling in a given region.

In this study we were interested in the prediction of the concentration of an element in the soil, given the concentration in the plant.

(a) normal distribution

This type of distribution is called 'normal' only because it was originally thought that it was the usual type found in nature. The mathematical model expressing the relative frequency (Z) of observations occurring at a standardised value x is given by the equation; $z = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x' - \mu}{\sigma}\right)^2}$

where x' is an observation value

μ is the mean of the sample and

σ is the standard deviation

The expression given above for z is said to be the 'standard normal form' of the normal curve, and any normal curve can be expressed in those terms by the appropriate transformation.

The normal distribution in standard form is illustrated in fig. II.1(a). It has a mean of zero, standard deviation of 1.0, is symmetrical about the y-axis, and extends indefinitely in either direction. The area under the curve is 1.0 sq. unit.

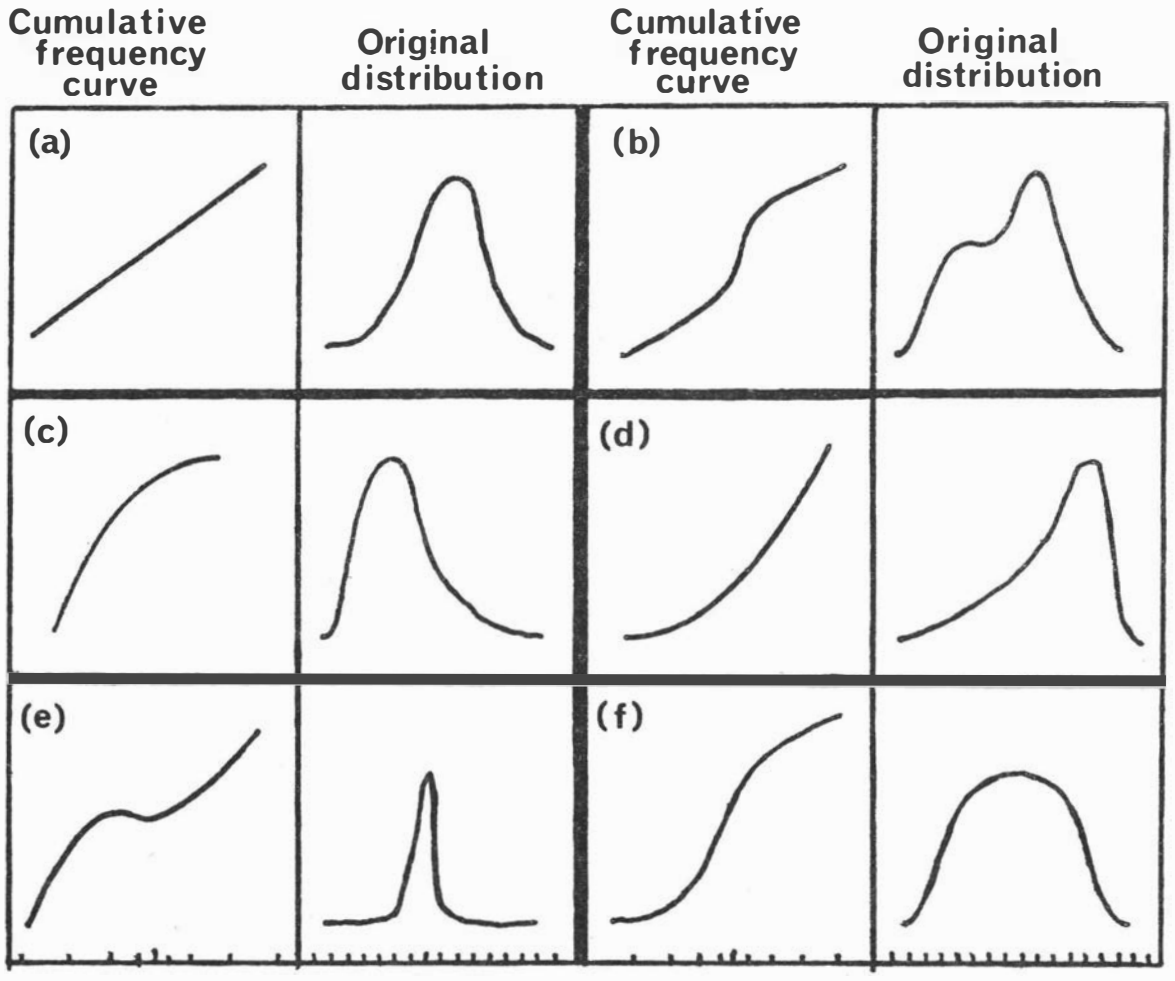
If any arbitrary value of x is selected, it is possible to predict, using the above mathematical model, the likelihood that any further observation will be greater than x. This is done by erecting an ordinate at the selected value of x, to cut the normal curve. The chance of a new value lying above the selected value is then given by the ratio of the area under the curve to the right of the ordinate to the total area under the curve, in this case 1.0. The probability is thus given by evaluation of the integral

$$P = \frac{1}{\sqrt{2\pi}} \int_{x}^{\infty} e^{-\frac{1}{2}x^2} dx$$

where the limit x is the selected value.

When the mean μ and the standard deviation σ are not known accurately and can only be estimated from small samples then $x = (x' - \mu)/\sigma$ follows the t- distribution. This is symmetrical about the y-axis and is rather flatter than the normal curve but approaches the normal curve closely as the sample size is increased.

A theoretical limitation to the normal distribution, as a model, should be noted. The distribution extends indefinitely in either direction. This should theoretically mean that there will always be a few negative values, even for data like soil analyses! Another limitation is that some sets of data skew, unlike the normal distribution. For these reasons the log-normal distribution, described next, may often be a closer approximation to reality.


Cumulative frequency curve
Original distribution
Cumulative frequency curve
Original distribution
(a)
(b)
(c)
(d)
(e)
(f)

(b) log-normal distributions

The log-normal distribution is so called, because the logarithms of the values are distributed normally rather than the values themselves. One example relevant to this thesis, is the observation by Dean, (1966) that uranium in the plants he studied was log-normally distributed.

This type of distribution is instanced in fig.II.1(c). It is a common type of distribution in geochemical and biogeochemical work, and Ahrens (1954) has gone so far as to suggest that trace elements in rocks always follow this pattern. This suggestion did not meet with universal approbation, and in the controversy which followed, further examples were produced, which either supported, or conflicted with the proposal. Rogers and Adams (1963) attempted to mediate by suggesting that the type of distribution was a function of the amount of the element present, and that only trace elements were log-normally distributed. Other workers, like Jurain, (1962b) concluded that for some elements, e.g. thorium, neither a normal, nor a log-normal distribution fitted the data well. However the model is sufficiently common, especially when trace elements are involved, that its use is justified, though it should be used with caution. If log-normal data are transformed logarithmically, the form then becomes normal and prediction as in the previous sub-section becomes feasible.

(c) other distributions

Many other types of distribution have been described. If data are even more skewed than those depicted in fig.II.1(c)., it is possible that the step of taking logarithms should be repeated in order to yield data approaching a normal distribution.

It is possible to imagine a case where the data are skewed in the opposite direction. For example, silica concentrations are high in most rocks, and we would expect analysis to reveal many high values, and few low values. The appropriate transformation here, might be the taking of antilogarithms. This type of distribution is instanced in fig.II.1(d).

Truncated distributions occur. In these, the distribution is incomplete, because some part of it has been removed. An example of this might be given by the particle-size distribution of a ground rock passed through a sieve. Ideally all particles below a certain size are removed and the distribution is truncated. In practice the cut-off is rarely sharp, but the important principle which remains, is that the mathematical model still holds within certain limits, and prediction is still applicable.

Other types of distribution include those described by Gaddum (1945) where a transformation of the type $X=\log(x'+x_o)$ with x_o a constant, normalises the data, and that described by Pearce (1945) where the appropriate transformation is $X=\sqrt{x' + \frac{1}{2}}$.

(d) Mixed distributions

Distributions may be produced by a number of processes, each of which may produce values normally distributed, but with different means and standard deviations. The end result would appear as in fig.II.1(b)., with either a large or small degree of overlap. In either case interpretation is difficult. Even two apparent distributions may be merely one partially-truncated distribution, and even what appeared to be a completely normal distribution might be made up of many small, independent distributions. It might be argued that this is unlikely; but the possibility remains and

there is no way of telling whether this is indeed true. The only principle which enables a systematic treatment of the problem to be made, is that one distribution be assumed where there is no firm evidence to the contrary, and that hypotheses be kept as simple as possible. We do not needlessly multiply hypotheses.

For example, in biogeochemistry, if a distribution is symmetrical but markedly flattened compared with a normal distribution, we assume two distributions are present, rather than postulating a dubious process which has removed values from the centre of the original, approximately normal distribution.

2. Cumulative frequency diagrams

(a) uses

Examination of data to determine what type of distribution is present is normally made by means of a histogram. Although helpful in many cases, it is often very difficult, even given the above principle, to tell whether the data are closer to normality, or log-normality and whether peaks within the distribution are real or merely the result of few data.

Cumulative frequency diagrams show the form of a distribution rather more explicitly than histograms, and graph the values being measured, against the percentage of values falling below the individual observation values. This procedure would normally yield a curve, and the percentage scale is normally constructed in such a way that a normal distribution yields a straight line on plotting on a cumulative frequency diagram. (See fig. II.1(a).

If two normal distributions are present there will be a tendency for two intersecting straight lines to be produced, and this is a more sensitive indicator of the presence of multiple distributions than the preparation of a histogram.

The use of these diagrams is discussed more fully by Tennant and White (1959).

(b) interpretation

Herdan (1949) has given a good description of the interpretation of curves obtained from these diagrams, and the information is summarised in fig.II.1. With practice it is possible to tell almost at a glance, the types of distribution present. It will be noted that the type of skewness implied depends on the convexity or concavity of the slope. Distributions may also be symmetrical, but kurtosed as depicted in fig.II.1(e,f).

(c) limitations

The procedure is still a qualitative one. A line obtained by this procedure is seldom completely straight, and it is often a difficult decision whether a minor deviation should be taken as significant or not.

Intersection points do not necessarily represent a boundary between distributions. Overlap occurs, and for full examination of individual distributions it is necessary to separate them by a rather tedious method (Cassie, 1954), and determine the probability of any given result falling in one distribution or the other.

The labour of drawing these diagrams for a large number of data

sets, makes almost any other method attractive. In this work I have attempted to reproduce as many features of these diagrams as possible, by mathematical methods alone (section II.B.2.).

3. Use of data for prediction: relations between variables

(a) regression

We are seldom interested in merely one independent distribution at a time. Often by use of an associated distribution, prediction can be substantially improved. For example if a measure of a quantity labelled y, and another labelled x, are linearly related, then for any given y, we can predict a value of x which is far more precise than merely assuming the distributions are independent and guessing from the total range of x what the likely value will be. It is therefore very important to determine whether y is indeed proportional to x, and how well this proportionality holds. One method of fitting such a line of proportionality is called regression analysis.

In this treatment it is assumed that for any given value of y there will occur a range of values of x, which will be normally distributed around some mean. For example, given the concentration of an element in some plant ash, we might require an estimation of the concentration of that element in the soil. In the field, there would be a range of possible soil values for any given plant value. The regression line is so fitted as to minimise the sum of squares of distances of all points from it, when the distances are measured in a horizontal direction. This line will pass through the mean of each x-distribution and is also known as the "line of best fit".

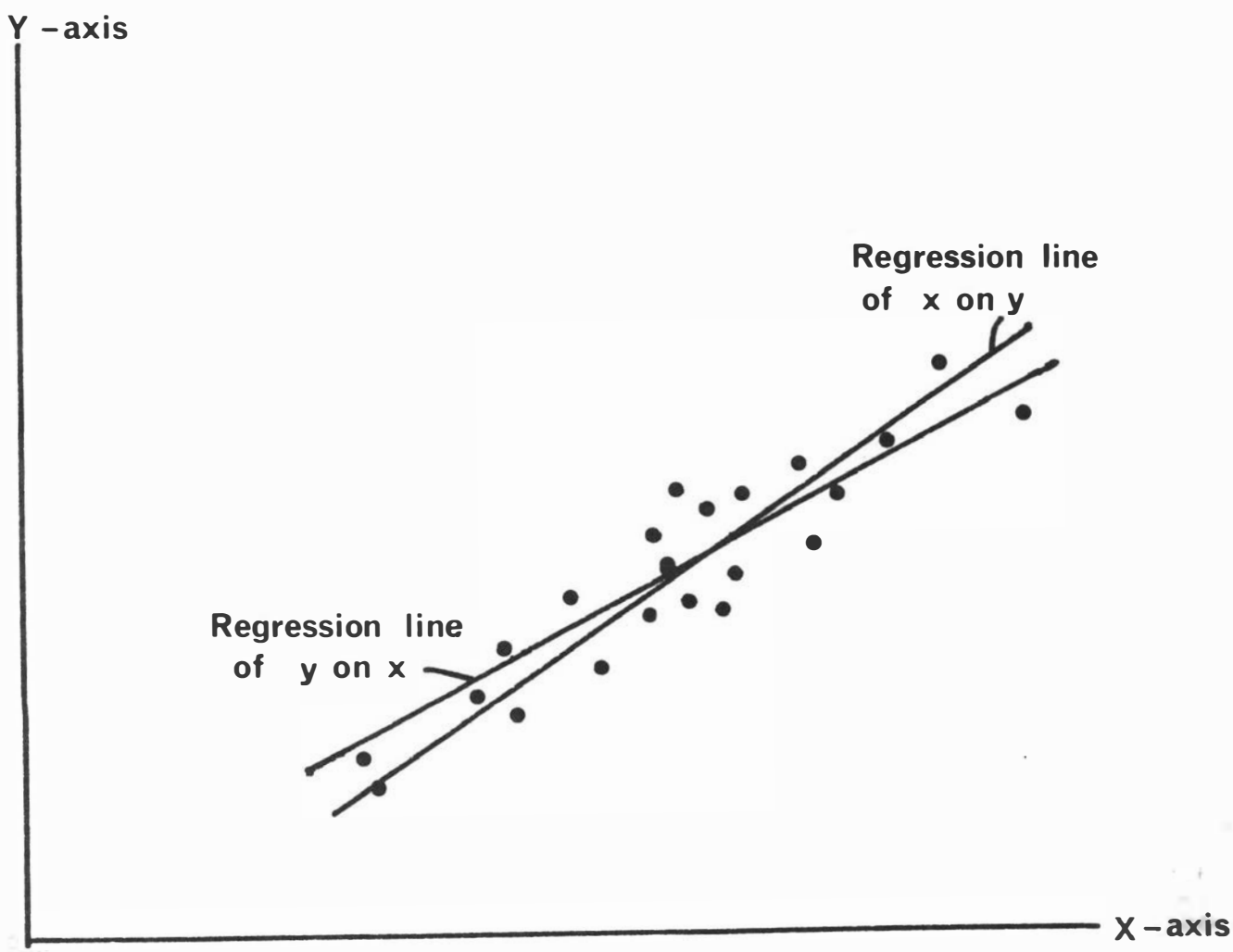
There is a tacit assumption here which often does not hold in practice. It has been assumed that for each distribution of x, y is known

exactly. What is usually found, is that both x and y vary at the same time, and to some extent are inter-dependent. For example, the concentration of an element in foliage certainly depends on the soil concentration, but through falling of leaves and soil formation from them, the soil concentration will also be partially dependent on the concentration of the element in the foliage. In other words, both x and y are normally distributed (at least within the limits of the observed values), and may be partially dependent on each other. In this case, there are two regression lines, of x on y as above, and of y on x, the latter calculated by minimising the sum of squares of vertical distances of the points from the line. Which line is used depends on which way prediction is desired.

(b) correlation

The correlation coefficient is a statistic universally given the symbol 'r' which expresses the degree of clustering of the points about the lines of best fit, and ranges from -1 to +1 (in either case the regression lines coincide, and all the points lie on the resultant line), through zero (in which case there is no relationship between the variables). The lines of best fit for a set of data are given in fig. II.2. The correlation coefficient for this set of data is positive, indicating there is a direct relationship between the two variables. A negative value indicates an inverse relationship, and a negative slope for the lines of best fit. The advantage of the correlation coefficient is that it does not assume independence of either variable, and force a choice of prediction in one direction.

If the data are not normally distributed, a very misleading value for r may be obtained. In one case in my experience, a correlation of


Y -axis
Regression line
of x on y
Regression line
of y on x
X -axis

0.92 was obtained for a set of data which contained two very high values. When these were removed and the calculation repeated, the value fell to 0.26, which was not significant.

B. Statistical inference from data

1. Correlation coefficients

The correlation coefficient is given by the equation

$$r = \frac{\Sigma xy - \Sigma x \Sigma y/n}{\sqrt{(\Sigma x^2 - (\Sigma x)^2/n)(\Sigma y^2 - (\Sigma y)^2/n)}}$$

Calculations on the biogeochemical data involved the summation of the individual observations, their squares, and the product of the x and y values for every pair of observations. Other statistics calculated for each set of data, were the mean, standard deviation, standard deviation as a fraction of the mean, mean slope of the lines of best fit, and the number of observations taken for the correlation. The mean slope of the lines of best fit was calculated from the ratio of the standard deviations of y and x.

2. Measures of normality

Since the calculation of correlation coefficients involved the use of the assumption that the data used were normally distributed, it was essential to determine for each set whether this was in fact the case. There are various methods of determining this, but it was necessary to choose a method that did not involve reordering the data, making correlation meaningless. The well known procedure of Fisher (1941) was used, during the course of which two statistics g1 and g2 must be calculated from the third and fourth powers of the data values. These statistics are approximately normally distributed with zero mean, and expressions for their standard deviations are also given by Fisher.

3. The significance of calculated statistics

(a) the correlation coefficient

Since x and y both vary, there is a finite possibility that the

ellipse of points in fig.II.2. should be a random scatter and that there is no real relationship between the two sets of data. Obviously the more observations in the set of data, and the tighter the points cluster about the fitted lines, the less likely it is that any observed relationship is by chance, and any evaluation of the significance must take these factors into account.

Evaluation of the possibility of r being zero, was done by the method of Fisher and Yates (1953). A statistic t, was calculated from r by the equation $t = r \times (n-2)^{\frac{1}{2}}/(1-r^2)^{\frac{1}{2}}$ which as its name suggests is distributed according to the t-distribution. The chance of t being significant, was then calculated in a similar manner to the treatment in section II.A.1. This involved the calculation of the area under the t-distribution curve, which is approximated by an expression quoted by Kendall and Stuart (1963).

(b) Normality measures

Since g1 and g2 are approximately normally distributed and their standard deviations are known, it was possible to calculate, using the methods previously described, the probability of either arising by chance. It was necessary to find the area under the normal curve to evaluate this, and I used the approximation to this integral given by Hastings (1955).

The volume of calculation for these significances is very large and required the use of a computer.

The approach of directly calculating the probability of any given result being due to chance alone, is rather unusual. Most workers prefer

to select a priori a level of probability at which they accept the idea that the statistic is not significantly different from some null value and refer to tables which give only the value of the statistic at that particular probability level. These tables usually contain probability levels of 0.1, 0.05, 0.01, 0.001, and the appropriate statistics underneath, but why should one be superior to another? It is well known that widely different levels of probability are accepted in different branches of Science, and even in the same branch. For example, in some psychological studies, a result may be accepted if the chance is as high as 0.1 that it is spurious; on the other hand, parapsychology is so suspect as a discipline, that investigators customarily choose probability levels of 0.000001 or even lower. The choosing of a probability level is highly arbitrary.

In industry, decisions about probability levels are facilitated by the management which often requires that not more than a certain risk be taken in terms of capital invested and other factors, but in biogeochemistry, such guidelines are not normally present, since a decision to commence drilling an area for evaluation of potential mineral deposits, is almost never taken on biogeochemical grounds alone.

Another disadvantage of a fixed probability level, is that tables are seldom complete, and rough interpolation may be necessary, making direct comparison of statistics very difficult. It is also possible that a result may be very highly significant, to such a degree in fact, that we can only say it is less likely to be spurious than the smallest probability given in the table; but it is often of real interest to know precisely what the probability is.

For these reasons I calculated the probability level for every statistic, and did not attempt to set an absolute cut-off point beyond which I thought the result to be probably zero. It may, in future, be possible on other grounds to set some such level, but at present it seems better to express all results on a relative scale. The system adopted here has the added advantage, that if such a future choice of probability level be made, the results can be accepted or rejected immediately by their associated probabilities, rather than by having recourse to a table.

4. Information gained by deviations from normality.

A surprisingly good idea of the shape of a distribution can be obtained by examination of the magnitude of the normality statistics, and their signs. (The magnitude of the statistic is preserved in essence, in the magnitude of the probability that it is real; to save print-out space the original sign of the statistic is printed in front of the associated probability, a practice which should not lead to confusion, since a probability itself can never take a negative sign.) If there is a high symmetry and the sign is positive the distribution resembles fig. II.1(c); if negative it will be as in fig. II.1(d). If there is a low degree of symmetry, the distribution will also be kurtosed. If the degree of kurtosis is high, then a positive sign indicates a distribution as in fig. II.1(e), and a negative sign a distribution as in fig. II.1(f), or more likely, two distributions, as in fig. II.1(b). If there is both high symmetry and low kurtosis then the data are normally distributed. Still more information may be obtained if the data are transformed into logarithms and the process repeated.

C. Computer programme

1. Selection of the optimum programme organisation

In section II.B.1. it was shown that for the calculation of correlation coefficients it is necessary to sum the x-y cross-products. This may be done automatically as the data are entered into the computer if one observation from each column is included. Storage locations are then set aside for cumulative totals of the data, their squares, and all possible cross-products. Very little final calculation is required, and the number of observations that can be fed in, is unlimited, since no permanent storage is required for the numbers themselves; only their sums etc. This type of programme organisation is ideal for small numbers of correlations, but suffers from three disadvantages. Firstly, data are entered in rows rather than columns, which means that the positions of the columns relative to each other, and their number, are fixed, unless one wishes to repunch every card. Secondly correlations with the data transformed to logarithms are complicated to obtain, and immediately double storage requirements. Correlations of logarithmic data against linear and/or vice-versa are very complicated to obtain. Thirdly if the number of columns is large, the amount of storage for cross-products etc., becomes rapidly prohibitive.

In spite of these disadvantages, the method has been successfully employed by Lyon (1969) on occasions where small numbers of correlations were necessary.

The second type of programme organisation, involves having all the data, permanently in core storage in the form of a matrix. Cross-products and other summations are calculated only as required, and their storage requirements can be much reduced. It is also possible to feed data

in by columns rather than rows allowing greater versatility. The disadvantages of this type of programme organisation are that the computation is invariably slower, and the total matrix size is limited by the core storage available. In practice I found it was not possible to store both an adequate programme, and a matrix containing 40 x 200 data points, even if the programme was subdivided and stored outside the core storage on a magnetic disk, calling into core only those fragments of the programme immediately needed.

The third type of programme organisation stores the matrix on the disk, and calls two columns of data into core at a time. Although this method still suffers from the disadvantages of the second type, and is even slower, the number of data points that can be accumulated is limited only by the very large capacity of the disk.

One feature of the data that initially caused some trouble was the fact that some plant elements are distributed normally when the elemental concentrations are expressed in terms of dry weight rather than ash weight, and there is hence the necessity to allow for correlations using both bases. This was achieved by creating two parallel matrices on the disk and performing all possible correlations on both.

Since some distributions are log-normal it was necessary to allow for the calculation of log-log, log-linear, and linear-log correlation coefficients. This was done as follows; Columns 1 and 2 were called into core, and column 1 was converted to logarithmic basis. It was then correlated against column 2, 3, 4, etc. until all possibilities were covered. Column 1 was then stored on disk (in place of the untransformed data) and column 2 was put in its place in core, transformed to logarithms and

correlated against columns 3, 4, etc. In this way, eventually all log-linear correlations are calculated, and the entire matrix transformed simultaneously into logarithms, and stored again on the disk. All possible log-log correlations may then be calculated, and by reversing the log-linear process, the linear-log correlations are accessible. After completion of the cycle the entire matrix will again be in the form of the original linear data. The entire cycle is repeated for the data expressed on a dry weight basis rather than an ash weight basis. The order of calculation is shown more clearly in fig. II.9.

Clearly, if the matrix is any size, an incredible amount of computation is required, hence options were included in the programme for calculating only those correlations required, e.g., log-linear, ash weight, log-log, dry weight. Options were also included to permit at any time during the programme, the calculation of either the correlation coefficients, the normality statistics, both, or neither, and the exitting from a given set of correlation calculations.

Another aid to versatility, was the provision of an option which enabled one column of data for correlation to be calculated from two data columns, by a process of either division, multiplication, or both. In the latter case, three columns were read and processed to give one column for correlation.

Finally, it was necessary to allow for missing data points in the matrix. For various reasons, a sample may be incapable of yielding an analysis figure. Thus in the execution of the programme, each data value

was examined, and if zero, that value and the corresponding value in the second correlating column were omitted from the calculation.

The programme was arranged in subprogrammes to enable easier debugging, and the easy transformation of the entire programme to load-on-call subroutines, if this is ever thought necessary. This inevitably meant some duplication of programme statements. The calculations were carried out on an I.B.M. 1620 - 40K Model II computer.

2. Flow diagrams

These are given in figs. II.3., II.4., II.5., and II.6.

3. Programme listing

As in fig. II.7.

4. A typical computation

(a) sample input

For execution of the programme, a number of cards other than data cards must be included. The following is the order of cards in the deck:

1. title card (which will be printed).
2. card designating incoming columns of data, and method of storage.
3. card giving length of column (maximum 200 at present).
4. data cards.
5. card specifying correlations required (e.g. linear-log, ash weight).

The title card may have characters to be printed on any column except the first.

An array of digits on the second card designates incoming columns as follows;

1. ash fraction (i.e. the fraction left of the original dry weight on combustion. This figure will be used to generate dry-weight basis data. In more general terms, any multiplier may be entered here.)
2. plant analysis figure (or any data requiring multiplication by 1.)
3. soil value. (or data not requiring arithmetical manipulation)
4. plant/soil ratio. (also requiring multiplication by ash fraction; this number stands for <u>two</u> incoming columns)
5. ratio not requiring arithmetical manipulation before storage. (Again specifies two columns.)
6., or greater, end of columns to be read.

These figures are listed consecutively from column 1 on the card with no spaces between them.

The third card carries the number of observations, right justified in the first four columns. No decimal point must be punched.

The data cards are punched with ten values per card, with each datum occupying a maximum of eight columns on the card. Provided a decimal point is included in the number, there is no limit on positioning within the eight spaces, but if no decimal point is punched, the number must be right-justified.

If the observations from one column do not fill the final card completely, the remainder must be left blank, and a new card started with a new column.

The card following the data, contains a series of digits separated by a single space. These specify the order and kind of the correlations required;

1. linear-linear, ash weight basis
2. linear-linear, dry weight basis
3. log-linear, ash weight basis
4. log-linear, dry weight basis
5. log-log, ash weight basis
6. log-log, dry weight basis
7. linear-log, ash weight basis
8. linear-log, dry weight basis
9., or greater, end of correlations to be computed.

By punching the digit three or four twice in succession, it is possible to convert the appropriate matrix to the logarithms of logarithms, and by putting the number seven or eight twice in succession the data may be transformed to antilogarithms. Though possible, these transformations seem unlikely to be extensively used.

The sense switches must be set as follows:
sense switch two on, the normality statistics are calculated;
sense switch three on, correlations are calculated.
If either is off, the appropriate statistics are not calculated.
Sense switch four on, the correlations are interrupted and the next set begun.

These switches may be operated at any time during execution of the programme.

The input cards for a demonstration run are given in fig. II.8.

(b) Sample output

In the running of this set of data, for demonstration purposes, the first six of the correlation options were designated for calculation. After the correlation of the first column with all the others, the sense switch controlling calculations of the normality statistics was turned off, since examples of them all were then present. After the completion of the linear-linear dry-weight calculations, the third sense switch was also turned off, to avoid the calculation of options three and four, which can be seen from the print-out to be pointless. The second and third sense switches were turned on again when the title for option five had been printed. The process was interrupted during the calculation of option six by the fourth sense switch which caused the programme to exit and seek further data for computation.

The print-out is given in fig.II.9.

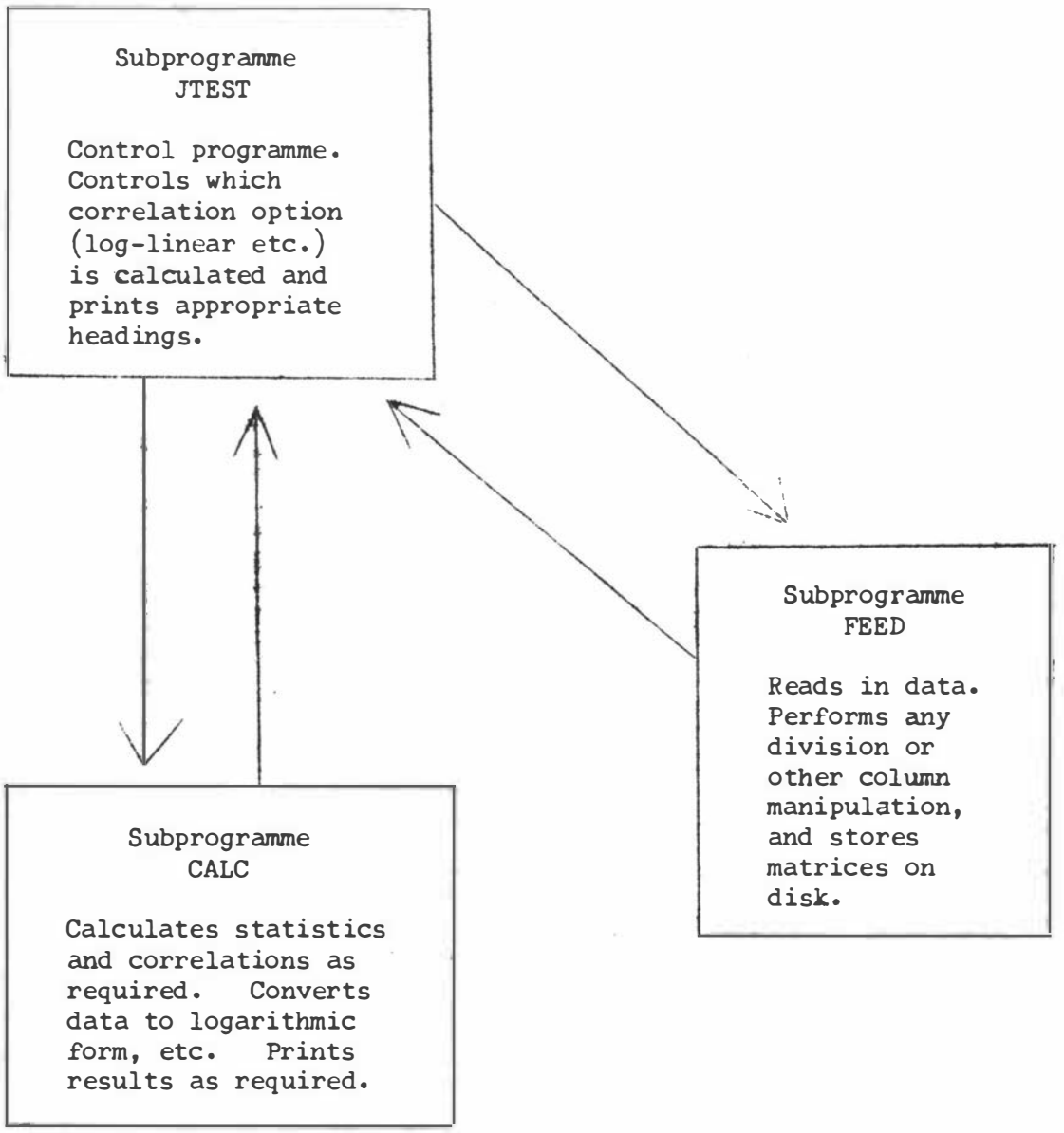


Figure II.3. Total organisation of subprogrammes within main computer programme.

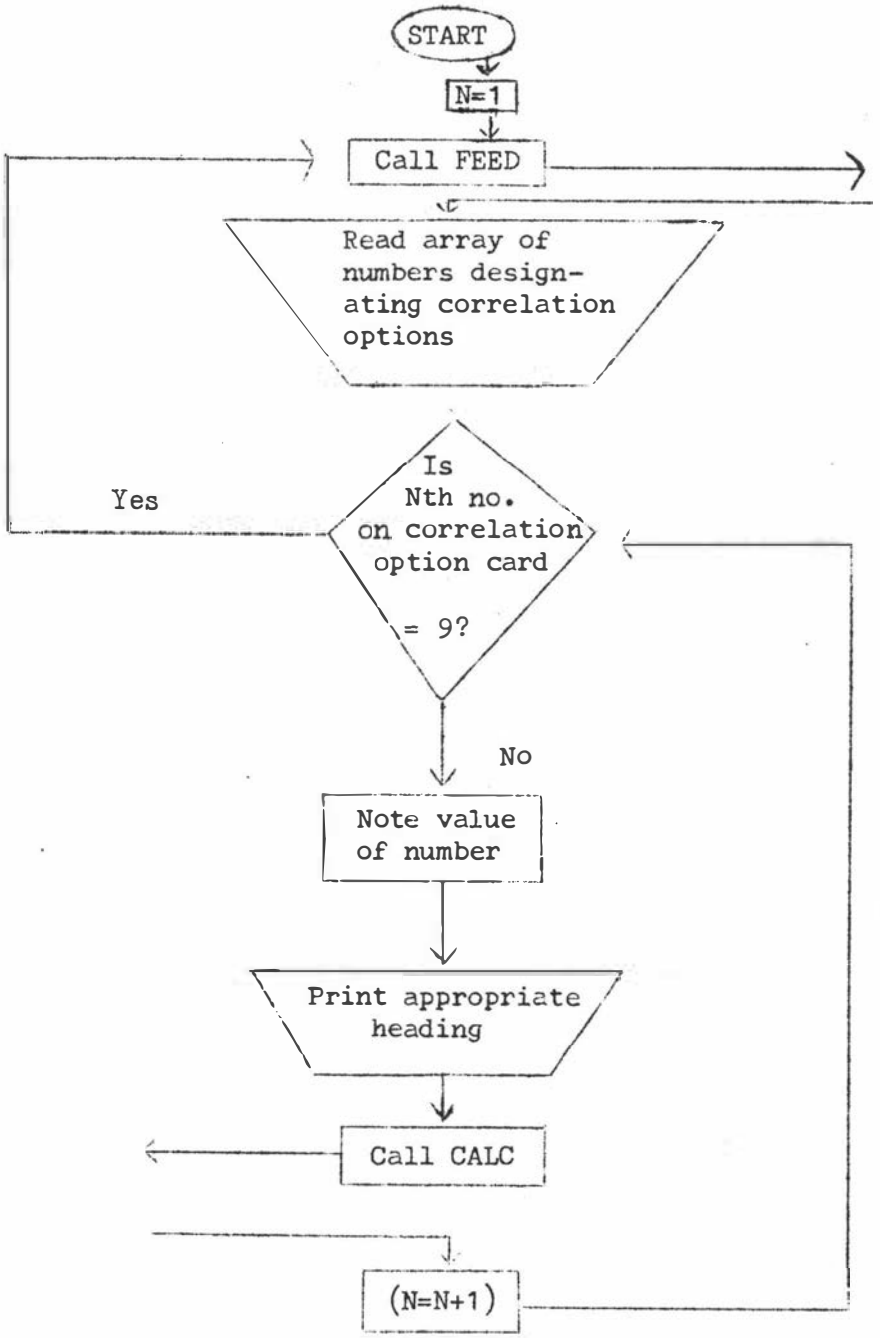


Figure II.4, Flow diagram for subprogramme JTEST.

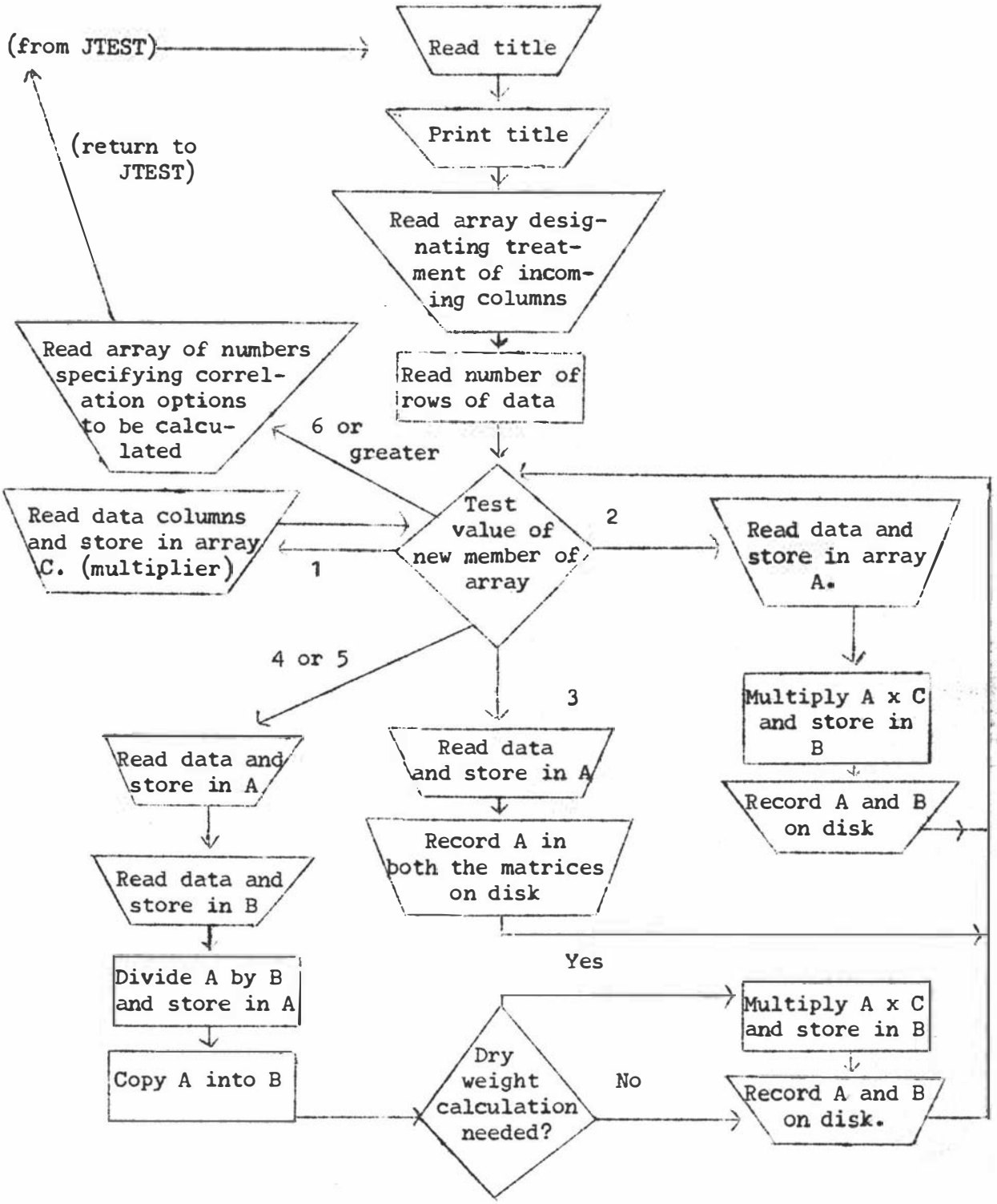


Figure II.5. Flow diagram for subprogramme FEED.

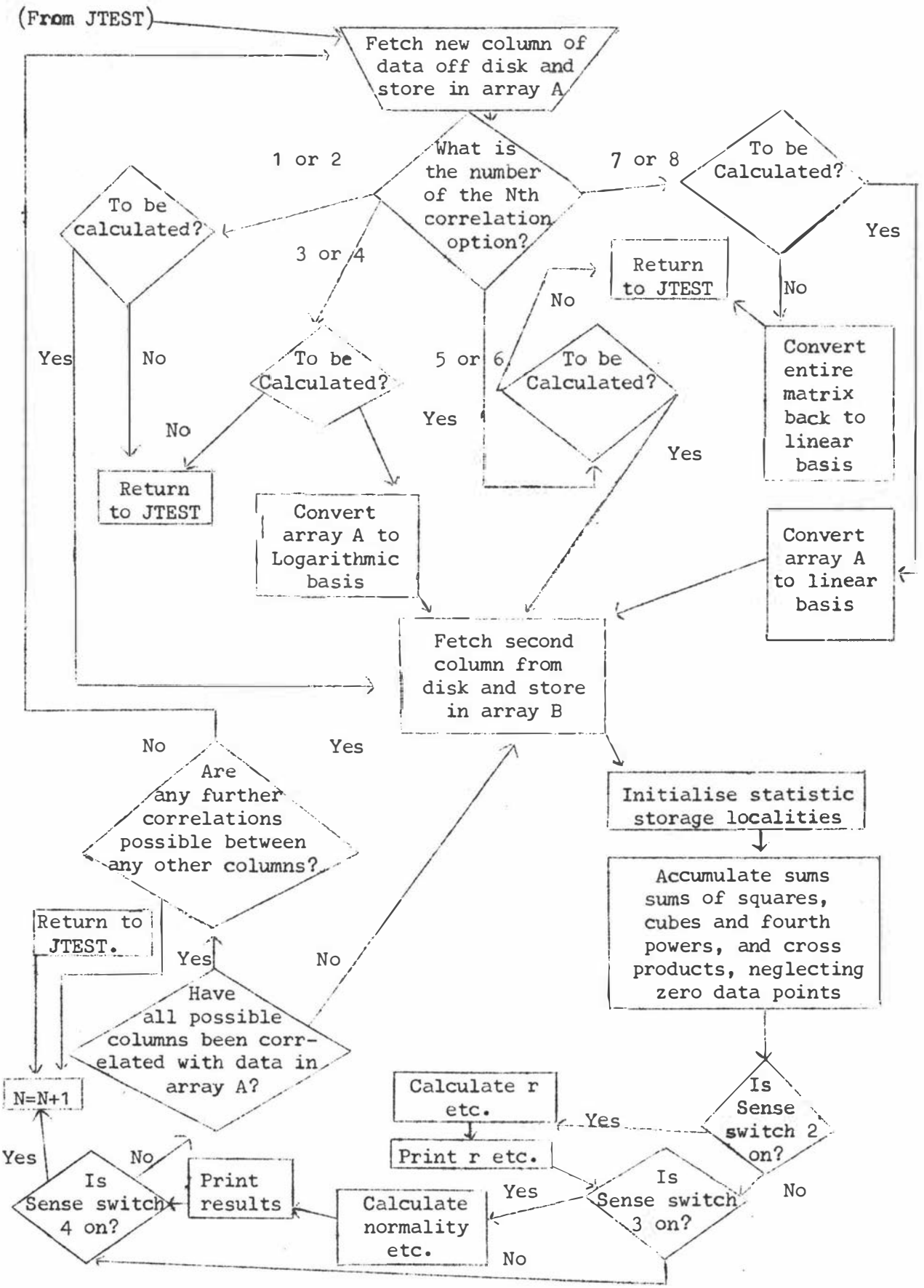


Figure II.6. Flow diagram for subprogramme CALC.

SECTION III

BIOGEOCHEMICAL SURVEY OF A MINERALISED AREA

A. Introduction

Biogeochemical prospecting for uranium was first introduced by Cannon and her associates in the United States (Cannon, 1952; Cannon, 1953; Cannon and Kleinhampl, 1956; Cannon and Starrett, 1956; Cannon, 1957; Cannon, 1959; Cannon, 1960a; Cannon, 1960b; Cannon, 1964; Kleinhampl, 1962; Kleinhampl and Koteff, 1960; Froelich and Kleinhampl, 1960).

Many of the deposits studied contained vanadium and selenium in conjunction with the uranium, and plants were found growing in the area which had a requirement for selenium in their metabolism. It was thus possible to use these plants as indicators of the presence of mineralisation containing selenium and hence, indirectly, the uranium ores. This geobotanical approach seems to have been rather successful. One of the findings was that high mineralisation induced morphological abnormalities.

The biogeochemical approach was also used, and of course involved the analysis of plant material for uranium. Somewhat arbitrary levels in the plant were selected to indicate mineralisation, and this technique was also very successfully probably because the Colorado Plateau deposits are in a semi-arid region, and the vegetation tends to possess very extensive root systems, which potentially can detect ore bodies, or their mineralised haloes, as deep as 70 ft. Care had to be taken with the selection of samples, since the amount of uranium varied with the season, the age of the plants, and even the side of the plant sampled. The amount of uranium also varied among plant organs with roots containing more than leaves. The problem of contamination was examined and found to be highly significant in the region of recent mine workings. The background level of uranium in plants was found to be about 0.5 ppm, and mineralised ground was taken to be represented by 1.5 ppm uranium

in the plant.

In addition to the above methods, an alpha-scintillation technique was developed by Anderson and Kurtz (1955) depending on the amount of alpha emission from plant ash being a measure of the amount of uranium in the soil. The method was claimed to be sensitive to 10 ppm uranium in the plant ash, below which the uranium emission was masked by the natural radiation of the sample. This natural radiation was probably fall-out products from the nearby Nevada test site, since the only major emitter of radiation in plant ash, ^{40}K, emits not alpha particles, but beta particles. Goldsztein (1957) however claims to have used a beta emission technique to successfully delineate uranium mineralisation at Esterel in France.

Rather later than the above work, a number of papers by Soviet authors appeared. Possibly because of early Russian work on the subject, (Brunovskii and Kunasheva, 1935; Kunasheva, 1944; Baranov and Kunasheva, (1954), radium, was usually measured concurrently with uranium, being a pathfinder for the latter. The usual assay for radium is a radiometric one depending on the outgassing of radon from the sample, its collection, and the recording of the alpha count rate.

Moiseenko (1959) developed the use of the biogeochemical method for uranium in swampy areas (see section IV for a fuller description of what this entails), and confirmed that the amount of uranium in plant ash was usually lower than the amount in soils.

Sokolova and Khramova (1961), and Kovalevskii (1962) in work which added little to the results of Cannon and others, used the method in various

parts of the Soviet Union and, as Cannon had done, observed abnormal forms of plants in uraniferous areas, and developmental retardation. They did not always find a very good correlation between the radioactivity of the plant samples and the uranium content.

Botova et. al. (1963), and Konstantinov (1963) confirmed other findings of Cannon, namely, that uranium tended to accumulate in the roots and wood of plants, possibly because of the relative age of these portions of the plant, since Yakovleva had confirmed (1963), that uranium accumulates with age. Once again the correlation between the measured radioactivity and the uranium content, was not very good.

Grodzinskii and Golubkova (1963, 1964) studied the nature of radioactivity in various plant and soil specimens, but did not reach any specific conclusions as regard to the alpha emission and isotopes responsible for it. Further studies (Kovalevskii, 1964; Kovalevskii, 1965) showed that radium is absorbed more than uranium, but the possible significance of this was not commented on. It was possible that daughter products of radium were contributing the bulk of the alpha counts. This finding was contrary to that of Anderson and Kurtz, and others in the United States who had shown that very little alpha radiation came from radium and thorium, but was possibly due to differences in the relative uptake of radium and thorium in the different plants studied in the two continents.

Makarov (1965) seems to have used the alpha emission of plant ash as a direct measure of the radium in the soil and assumed that 'haloes' of radium were formed around a uranium deposit, in the same way that a halo of uranium itself is found. The method seemed to be successful, but it relied

on the doubtful premise that the alpha emission was due partly or mainly to radium and daughter products. What he may in fact have been measuring was the emission from uranium in the leaf ash, and this would naturally give a good indication of whether there were uranium in the soil or not!

The American and Russian workers have contributed by far the bulk of the papers on this subject, but a few other applications of the method also are worth noting. Debnam (1955) in Australia, found that various local plants were useable in the biogeochemical method, and that the leaves usually contained higher amounts of uranium than the roots. This difference from the American workers suggests that this type of localisation may be more typical of evergreen trees, or other metabolic factors may be important. Lecoq et al. (1958) and Ohashi and Murakami (1960) have also applied the method in tropical Africa and Japan, respectively.

Finally, Mamulea and Buracu (1967) in their use of the method in Roumania, found copper was associated with the uranium, and suggested that the copper was absorbed by the plants and this somehow provoked the uptake of uranium, though it is difficult to see a mechanism for this, and the authors do not provide one.

There were thus some discrepancies in the literature which seemed worth investigating, especially the nature of the alpha counts and the best part of the plant to sample. It was also necessary to check under the conditions found in New Zealand if plants actually were useable in biogeochemistry. Of necessity this problem must be faced afresh for any new area whose vegetation differs markedly from those already investigated.

The study described in this section was intended to be only an orientation survey, for possible use in future surveys in unknown territory, and was an attempt to compare a biogeochemical prospecting method with other methods which had been employed on the area of mineralisation.

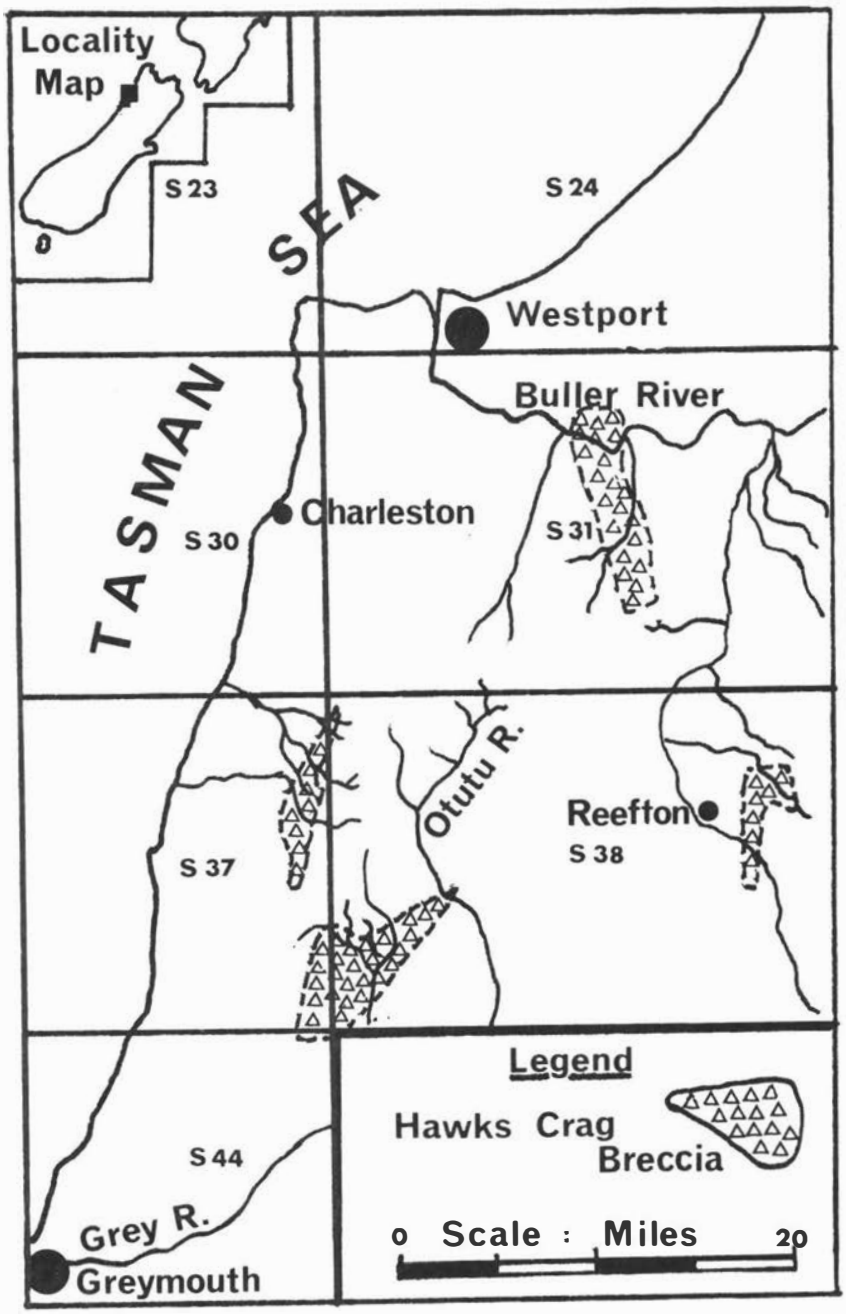
B. Description of area studied.

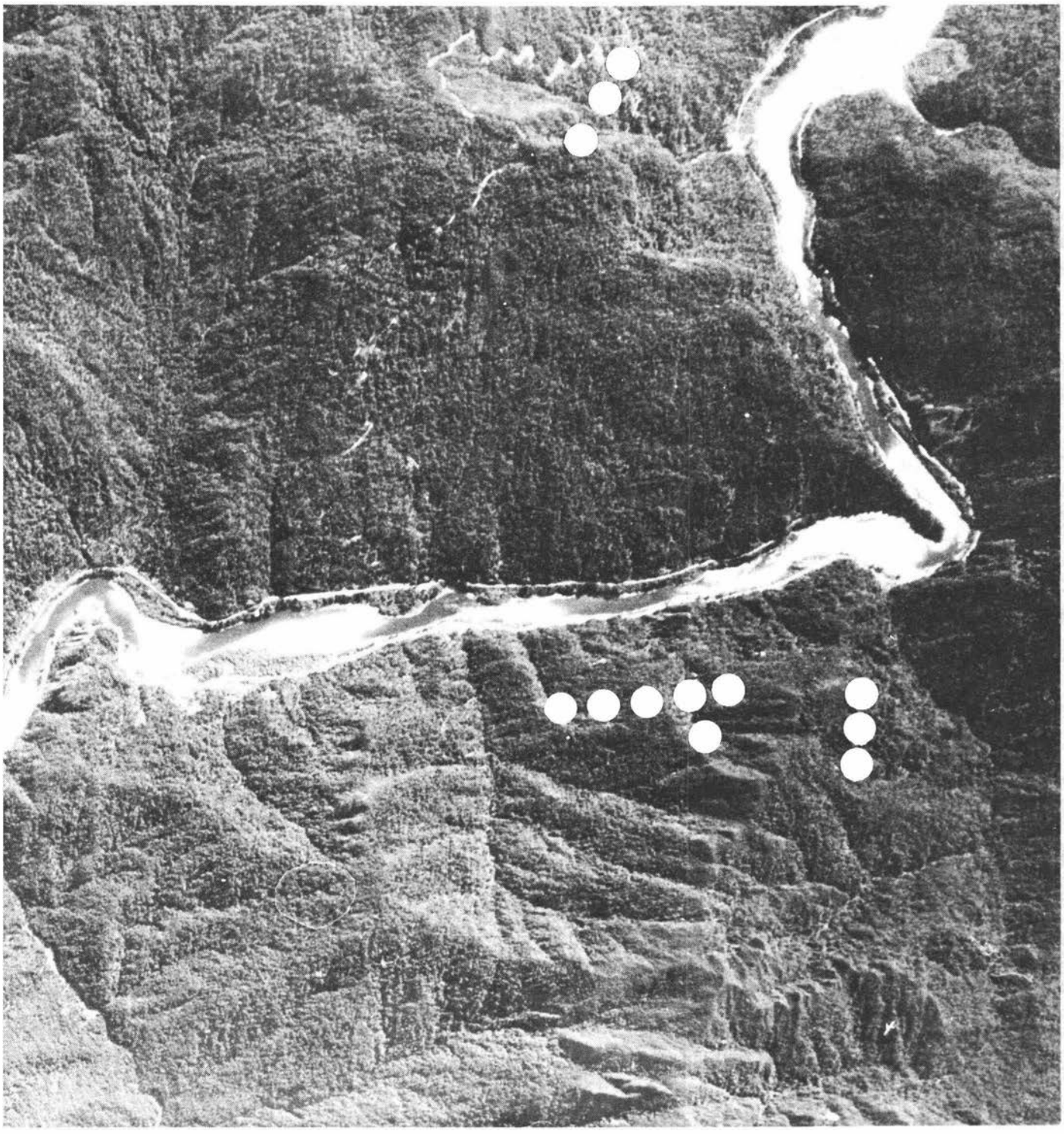
1. Physical features

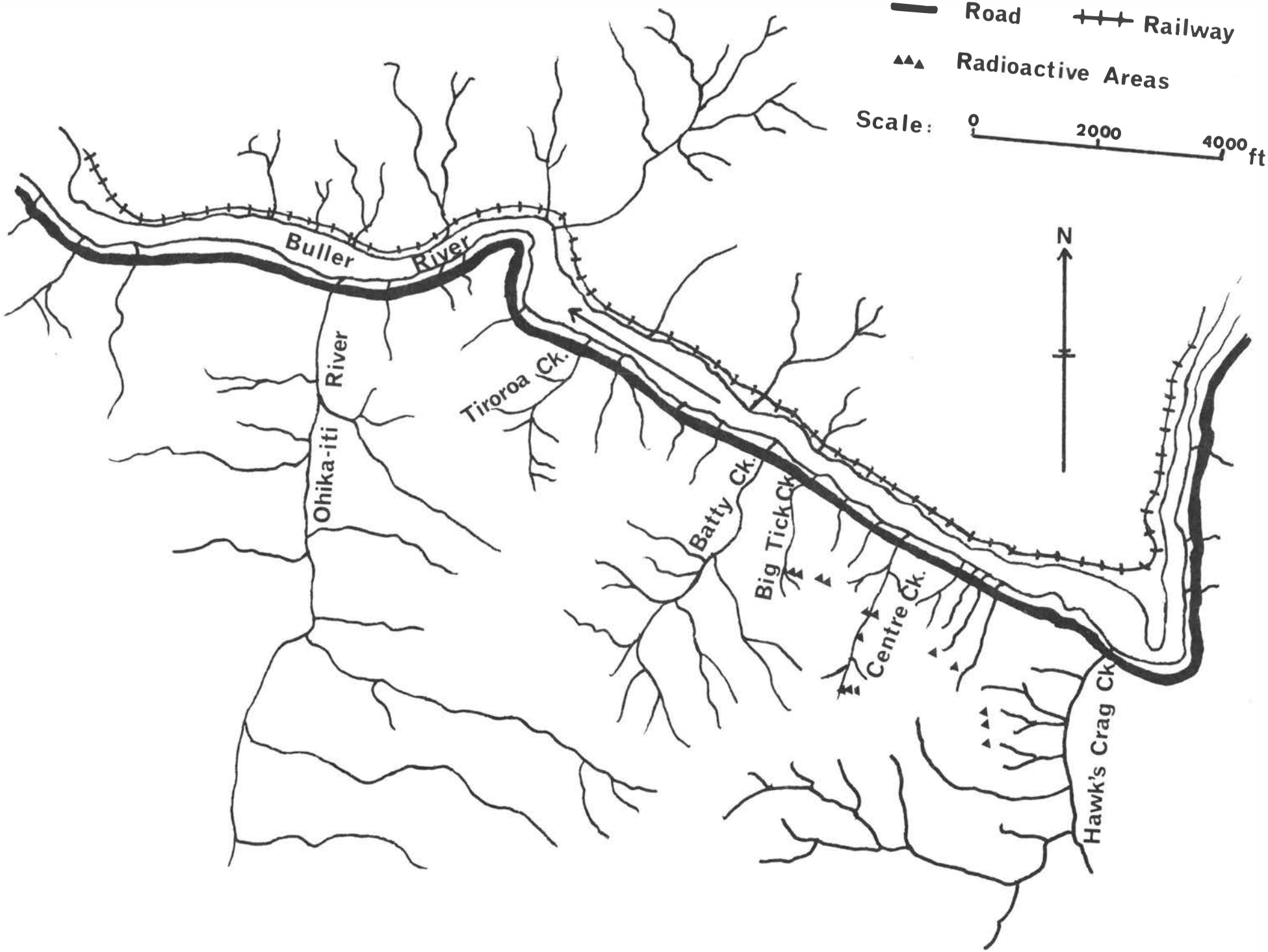
The area of uranium mineralisation is located near the Buller Gorge on the West Coast of the South Island of New Zealand, and is some 17 miles east of the town of Westport (see fig. III.1.). The topography is illustrated in plate III.1. and is typically very steep and broken. Waterfalls are very numerous, making travel by creeks very difficult, and access to the mineralised area is mainly by ridges. The access route and positions of mineralisation are shown in fig. III.2., and plate III.2. Fortunately a road runs through the Buller Gorge, and the deposits can be reached from it in about an hour.

There is erosion in some areas, due to rather frequent earthquakes (causing slips), and a high rainfall of 150 inches per year. Skeletal soils are found on the steeper slopes. In the more stable parts of the terrain, the soils have been classified as moderately-weathered podzolised, yellow-brown earths, with pH values ranging between 4.5 and 6.5 in all horizons.

The high rainfall makes the region very different from most others studied. Plants have relatively shallow roots, but are still good indicators of the presence of mineralisation because of the formation of relatively large mineralised haloes in the soil around the deposits. The soils are taken as being good indicators of the presence or otherwise of mineralisation, and the plants are useful only in-so-far as they reflect the levels of mineralisation in the soils.


Locality
Map
S 23
S 24
TASMAN SEA
Westport
Buller River
Charleston
S 30
S 31
Otutu R.
Reefton
S 38
S 37
S 44
Grey R.
Greymouth
Legend
Hawks Crag
Breccia
0 Scale : Miles 20




Road
Railway
Radioactive Areas
Scale: 0 2000 4000 ft
N
Buller River
Ohika-iti River
Tiroroa Ck.
Batty Ck.
Big Tick Ck.
Centre Ck.
Hawk's Crag Ck.

2. Vegetation

The whole region is covered with a dense beech and podocarp forest typical of this part of New Zealand. In the alluvia of creek bottoms, Nothofagus menziesii and Weinmannia racemosa are common, although Nothofagus fusca occurs extensively in similar areas with slightly better drainage. On the skeletal soils found on the steeper slopes and ridges, the main species are N.truncata, W.racemosa and Metrosideros umbellata. M. umbellata becomes increasingly common on steeper ridges, whereas on the flatter ridges there is local dominance of Dacrydium cupressinum. At higher altitudes there is an increasing abundance of N.menziesii with much M. umbellata and Podocarpus hallii. All the above species are trees of considerable size, but beneath the forest canopy extensive growth of smaller trees and shrubs is found. The commonest species are Quintinia acutifolia, Coprosma australis, Myrsine salicina, Carpodetus serratus, Pseudopanax spp and Brachyglottis repanda.

The denseness of the vegetation poses peculiar problems. Soils are very difficult to find and sample, hidden as they usually are, underneath a layer of humus, and many of the trees which are potentially most useful, have leaves which are inaccessible for sampling, because the specimens are so tall. This limited to a surprising extent the total number of one species that could be sampled.

3. Mineralisation

The geology and mineralogy of the Lower Buller Gorge uranium deposits, have been described by Morgan and Bartrum (1915), Wellman (1950), Beck et. al. (1958) and Whittle (1960). The descriptions which follow have been taken from these authors.

The basement rocks of the area include Paparoa granite-gneiss, which varies in composition from granite to granodioritic, Greenland series sediment, and quartz-porphyry intrusives. Gneissic banding is common, and in some localities, the gneiss is porphyritic.

Resting unconformably on the basement rocks are the Greenland series, which consist of well-indurated phyllitic greywackes of Paleozoic age, and the Ohika beds (Upper Jurassic) which are non-marine shales, tuffs, sandstones and conglomerates.

Resting unconformably on the Ohika beds is the Hawks Crag Breccia (Middle to Upper Cretaceous) which has been divided into three facies, depending on the relative amounts of parent material present. These are (i) the Tiroroa facies, consisting of rounded to sub-rounded granite pebbles, and boulders which are set in a coarse, angular, arkosic matrix, (ii) the Dee Point facies and consisting of angular to sub-angular greywacke fragments set in a silty matrix, and (iii) Blackwater facies, consisting of granite and greywacke pebbles and boulders set in a silty matrix.

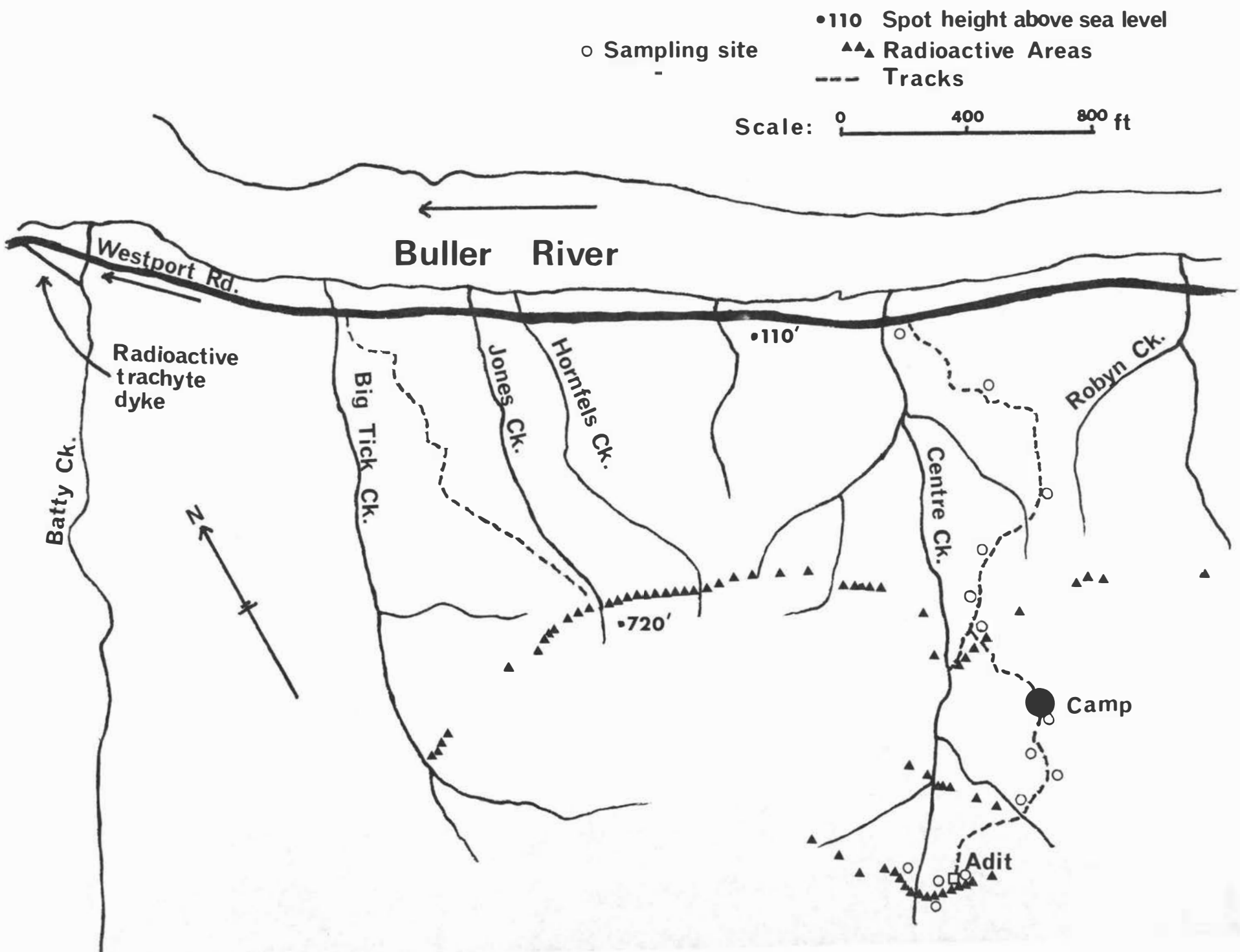
The ore bodies occur within the Hawks Crag Breccia, and apart from an absence of selenium and vanadium, bear a resemblance to the Colorado Plateau deposits in the United States. The deposits are stratiform and their thickness and areal extent varies in much the same way as those of the Colorado Plateau.

There is no obvious relationship between rock structure and ore deposition except in a few instances where mild shearing and microfolding has localised carbonaceous radioactive material. Fossil woody material has been carbonised and partially replaced with uraninite, together with sulphides of iron and copper. Some coffinite is also found. Secondary minerals found by Whittle (1960) include autunite, gummite, and torbernite. Cohen et. al. (1969) were able to show that copper, lead and beryllium were correlated with uranium in stream sediments, soils and minerals, and were thus useful as pathfinders; but zinc, thorium and the rare earths could not be used in this way.

One possible reason for the relative lack of thorium and the rare earths, is that the uranium has been selectively leached from some other area and deposited relatively recently. This is partially supported by radiometric measurements by Coote et. al. (1970) which show that the amount of radium present is smaller than it would be if sufficient time had elapsed for equilibrium.

The mean activity at the area of mineralisation was about 50 microroentgens per hour but at places was higher than 1 milliroentgen.

During 1958, a shallow exploratory adit was driven at the location marked in fig. III.3. but with the high rainfall preventing dust formation and the intervening period of time it is not thought that significant contamination occurred.


•110 Spot height above sea level
Sampling site
Radioactive Areas
Tracks
Scale: 0 400 800 ft
Buller River
Westport Rd.
Radioactive trachyte dyke
Batty Ck.
Big Tick Ck.
Jones Ck.
Hornfels Ck.
•110'
Centre Ck.
Robyn Ck.
N
•720'
Camp
Adit

C. Orientation survey of a mineralised area.

1. Selection of suitable plant species

Plant samples were collected as detailed in section I, and were thoroughly washed before ashing. Analysis for uranium, was done by the previously described method and the alpha and beta counts were also recorded. The associated soils were similarly analysed.

The beta count did not prove to be of great value. Fig. III.4. shows that the beta counts for plants tended to decrease to a constant level whereas the alpha counts decreased continuously. This is almost certainly due to the natural ^{40}K in the plant ash, so after this initial finding, beta counting was suspended in favour of alpha counting alone.

Table III.1. gives a list of the most common plants in the area of high mineralisation with mean uranium figures, alpha counts, and plant/soil ratios. In most cases, with the notable exception of the fern Blechnum capense, the amount of uranium in the plant ash is much less than the amount in the soil. The amounts of uranium in the soil vary, but are very high since the area of mineralisation was very close. This is specially marked in the case of the soil of the specimen of Uncinia leptostachya which was practically ore grade, and the plant itself contained the astonishing amount of 2.5% uranium in the ash. This would seem to be the highest figure yet recorded for uranium in a plant ash, but the finding is of little use other than of general interest, since the root system of this sedge is small and rather shallow. Other very high uranium contents were recorded for the moss Polytrichadelphus magellanicus, and the lichen Stereocaulon ramulosum. Both these were growing about three feet below a uraninite vein in a cliff, and were constantly bathed in a film of water which dripped down over the uraninite and onto them.

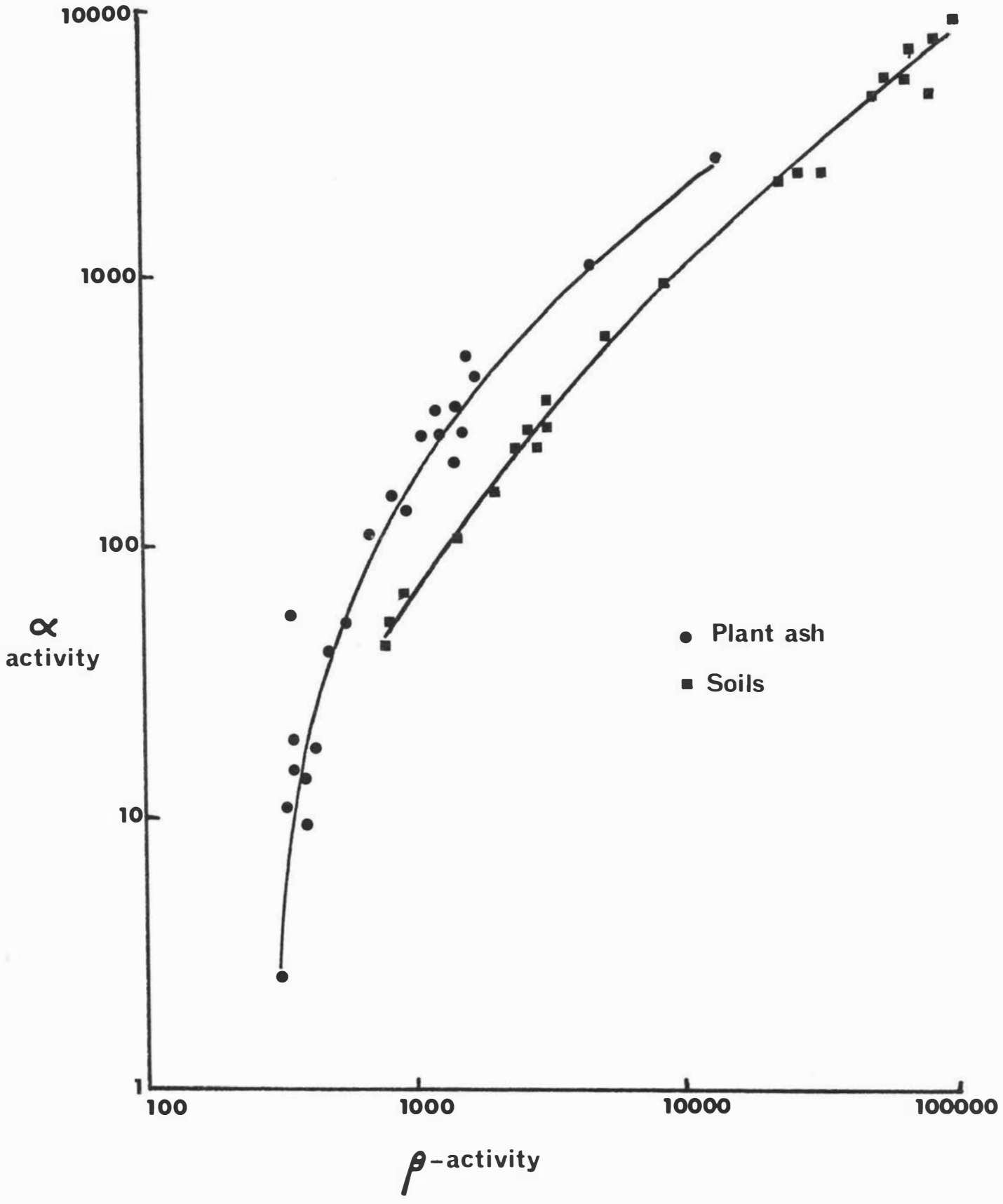

10000
1000
100
10
1
α activity
100
1000
10000
100000
β-activity
Plant ash
Soils

Table III.1.

Relative accumulation of uranium and alpha activity by plants of the Buller Gorge region.

Sample	Mean activity (counts/100 min/50 mg plant ash or /125 mg soil)				Mean uranium content (ppm)		
Bryophytes	No. of samples	Plant	Soil	Plant/ Soil	Plant	Soil	Plant/ Soil
Marchantia berteroana	1	7108	8288	0.86	670	4900	0.14
Polytrichadelphus magellanicus	1	16913	-	-	4360	-	-
Stereocaulon ramulosum	1	3913	-	-	1420	-	-
Ferns							
Blechnum capense	1	4696	2882	1.63	705	715	0.99
Dicksonia lanata	1	4608	4772	0.98	238	3280	0.07
Monocotyledons							
Cordyline banksii	1	478	1086	0.44	18	12700	0.001
Uncinia leptostachya	1	19086	21678	0.88	25100	36500	0.71
Dicotyledons							
Carpodetus serratus	6	745	7310	0.10	291	8616	0.03
Coprosma arborea	1	122	37520	0.03	987	30200	0.03
Coprosma australis	6	1500	5000	0.30	150	3000	0.05
Cyathodes fasciculata	1	836	5150	0.16	495	63800	0.008
Myrsine salicina	2	110	315	0.35	68	23850	0.003
Nothofagus fusca	6	30	30	1.00	20	100	0.20
Pseudowintera colorata	1	244	2122	0.11	26	2045	0.01
Quintinia acutifolia	6	600	600	1.00	30	200	0.15
Weinmannia racemosa	4	80	500	0.16	20	100	0.20

It is quite remarkable that these plants seem to have the ability to accumulate amounts of uranium that would be expected to cause toxic effects and a brief search was made for these. The U.leptostachya was growing in a rather marshy area, and a return visit after six months showed that it had spread considerably. Apparently normal seeds were produced, but no attempt was made to germinate these. Some specimens of Q.acutifolia had purple blotches on their leaves, which appeared similar to those observed in section VI, when high levels of uranium were fed to specimens of C.australis. However these patches of strange coloration were not noticed in the field specimens of C. australis; only chlorosis and necrosis (Plate III.3) in plants from very heavily mineralised ground. No abnormalities of form were seen, which might aid in geobotanical prospecting.

From the study above only five species were found, that grew commonly in the mineralised area; Carpodetus serratus, Coprosma australis, Nothofagus fusca, Quintinia acutifolia and Weinmannia racemosa; whereas there were many other species which did not seem to grow in the mineralised area to any extent. This might have been partly due to the disruption of vegetation by the previously mentioned exploratory adit, but limited severely the number of species that could be used, since comparison in mineralised and non-mineralised ground was important. In view of the disturbance of the area it was not really possible to say whether the absence of these other species represented an aversion to mineralised ground, and hence a geobotanical study was not feasible. It would be necessary to study an undisturbed area, and this would be very difficult on account of the thickness of the bush. The best way to study the distribution of vegetation in areas such as the New Zealand bush would be from the air, preferably using infrared

colour film, which differentiates various occurrences of chlorophyll, much better than normal colour photography.

The five selected species could not be sampled on a grid pattern because of the extreme roughness of the topography. The method adopted was to sample plants and their associated soils and determine the degree of correlation by the methods outlined in section II. Soils took about three times longer to collect than plant samples. The main plants selected are illustrated in plates III.4., III.5., III.6., III.7., and fig. III.3. shows the main sampling sites except for those plants selected as background specimens. The sampling sites were all down-hill from the mineralisation. On an average only 25 plants were selected for each species, which was due mainly to the lack of suitable specimens in or near the mineralised area. This may seem a very small number indeed, but since such a wide range of uranium values is covered, and the statistical treatment allows for the small sample, it is not serious.

2. Localisation of elements in various tissues

One specimen each of C. australis and N. fusca was analysed to determine whether one part of the plant was markedly superior to another for sampling. In the case of C.australis, a shrub, it is possible to uproot the entire plant in the field, so all portions were analysed. N.fusca attains a great height and leaves are often inaccessible, so the leaves, twigs and bark were analysed for only one mature tree.

The Coprosma was found growing two feet beneath a thin lense of uraninite. The surface of the cliff was continuously damp and water passed

over the uraninite before reaching the soil in which the Coprosma was found, giving the soil a scintillometer reading of 550 microroentgens per hour. The plant was carefully uprooted and very thoroughly washed. The roots were peeled and the peelings and remainder analysed separately.

The results of this analysis are shown in table III.2., and indicate that the root bark contains most of the uranium, with the root itself containing little, and with the amount decreasing from the leaves down to the roots. The figures in the table are expressed on a dry-weight basis, because the amount of ash of the various parts varied from 1.7 to 10.5%. They thus represent the relative amounts in the tissues in a more meaningful way than the results expressed on an ash-weight basis, and also indirectly show the relative amount of tissue that would have to be taken to obtain the same amount of uranium for analysis.

The Nothofagus specimen was a ninety-year-old tree felled for the purpose. The results in table III.3. are again on a dry-weight basis, and are also for a number of other elements. The leaves usually contain as much or more of the various elements than any other part of the tree, but in the special case of zinc, the bark appears very high indeed. It is uncertain how far these results may be extrapolated, but it seems that the procedure of leaf sampling is probably justified, and that bark sampling may be useful in some cases. The latter idea was not investigated further.

3. Normality of data

The plants were analysed for uranium, copper, iron, zinc, calcium, magnesium, potassium, sodium, and alpha counts were recorded. These elements were known to be either pathfinders for uranium or elements

Table III.2.

Total analysis of a specimen of C.australis

Part of plant	ppm uranium (dry-weight basis)
Leaves	60.5
Twigs	44.0
Main ste	15.4
Root	6.9
Root bark	106

Table III.3.

Analysis of parts of a specimen of N.fusca

	Elemental content (dry-weight basis)								
	Fe ppm	Cu ppm	Zn ppm	Mg %	Ca %	U ppm	Na %		K %
Bark	13.6	4.0	440	.0003	1.24	0.18	2.00	2.3	3.6
Twigs	4.9	1.0	2.9	0.03	0.195	-	0.036	0.9	0.031
Leaves	30	3.2	20	0.11	0.56	0.17	0.26	1.5	0.05

associated with trace element metabolism in plants. Beryllium was measured via emission spectrography, but when there was found to be no relation with other elements either in the plant or soil, the assays were discontinued.

The Coprosmas tended to be plentiful around the mineralised area which was relatively open, but rather rarer where the bush was denser, so this species was analysed only for uranium and alpha emission. Carpodetus serratus was first analysed for uranium and the results even at a cursory glance obviously bore no relation whatever to the amount in the corresponding soils. The reason for this unusual lack of relationship is difficult to understand but could be due to a fixation of uranium at root level leading to variable amounts in the leaves. The use of Carpodetus is hence not recommended for biogeochemical prospecting purposes, and the species was not further considered in this study.

Before other correlations could be calculated it was necessary to consider whether the data were normally distributed, since calculation of correlations is valid only to the extent this is so.

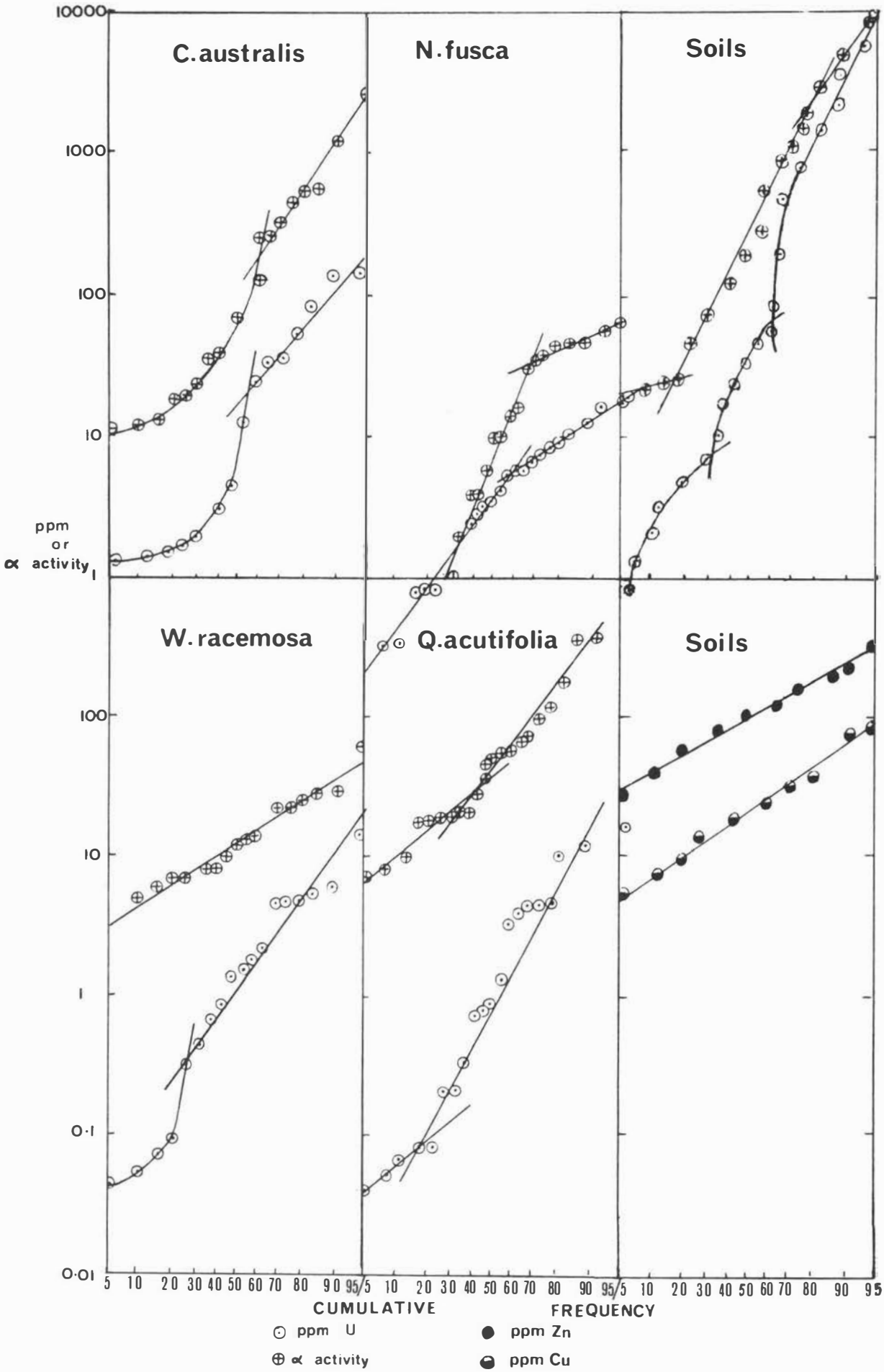
The calculation of the normality statistics gave answers which expressed the probability of symmetry or kurtosis being present, and the problem of choosing whether a given result was significant or not had to be faced. However the results were rather clear-cut in this case, and difficult decisions were postponed. Without exception, when untransformed data were considered, they were found to be neither symmetrical nor unkurtosed, with odds of 10^8 to 1 on average, which is indeed a rather clear

demonstration that some other type of distribution was representative of the data.

Transformation to a logarithmic basis gave rather mixed results. The odds became about 50 to 1 in favour of the data being symmetrical (with a few minor exceptions), but the odds were still very high against the data being unkurtosed. It would seem though, that a lognormal model for the distributions is superior to a normal model. From the treatment in part II, and the direction of the kurtosis, it follows that there are two distributions present, the data being too spread out to be a normal distribution. Only in the case of magnesium (N.fusca twigs, W.racemosa twigs) and zinc (Q.acutifolia leaves) and the alpha counts for all three plants did the sign of the kurtosis statistic show that the data were narrower than those of a normal distribution, and the only set of data which appeared to be normally distributed were the potassium figures for N.fusca leaves; it is difficult to tell whether or not this has any bearing on the good correlation with zinc and copper in the soil observed in subsection 5(c).

4. Cumulative frequency diagrams

Although the statistics just described give a good idea of the shape of distributions, and afford an objective criterion for decision as to whether there is more than one distribution they may still seem rather abstract. For this reason, a few sample cumulative frequency diagrams are now presented in fig. III.5. Although the zinc and copper figures have had a single line drawn through them, the statistics show two distributions are present. The total range of the zinc and copper figures is much less than that for the alpha counts and uranium.


C.australis
N.fusca
Soils
W.racemosa
Q.acutifolia
Soils
10000
1000
100
10
1
0·1
0·01
ppm
or
∝ activity
5 10 20 30 40 50 60 70 80 90 95
CUMULATIVE FREQUENCY
⊙ ppm U
⊕ ∝ activity
● ppm Zn
◒ ppm Cu

What do the multiple distributions in these diagrams represent?

One might imagine that the two distributions in the soil might represent mineralised and non-mineralised samples, but probably another mechanism is responsible. The uranium values for soils taken from regions known to be unmineralised were 1 - 1.5 ppm, and the inflection point on the graph is much higher, at about 80 ppm of uranium. Similar comments apply to the alpha counts. One possible reason for both the plant and soil multiple distributions, is found in the phenomenon of binding. Both soil and plant material very likely possess different types of binding sites for uranium, and when one is saturated the second begins to be filled, giving two or more distributions and the observed cumulative frequency diagrams. It is unlikely that any given distribution in the soil corresponds to one in a plant because for a cation to enter a plant it probably does so in a single chemical form, in the case of the uranyl ion, perhaps as a complex carbonate. If any of the uranium is bound in the soil as some insoluble compound it will enter the plant via a single route exactly as the more soluble form, thus tending to destroy the effect of the previous distribution. Once inside the plant, the distributions are determined by other factors like the amount bound at root level, and whether this mechanism reaches a saturation point.

5. Correlations

Since the data were not in general normally distributed even on a logarithmic basis, it might be imagined that the correlation coefficient would be completely meaningless, since it relies on the assumption of a normal distribution rather heavily. However this is not completely true, because the deviations from normality were mainly in the direction of wider distributions than are allowed for by the normality model, and since most

deviate in the same direction, and approximately to the same extent, the correlation coefficients are still useful as a relative measure of correlation, though the absolute values, and the absolute values of the associated probabilities that the correlations are real will have less meaning.

To find out which was the best basis for correlation, all correlations from individual sets were summed. That is, all the correlations from linear-linear, log-linear, log-log, and linear-log combinations were summed without regard to sign, ash and dry-weight bases being considered separately. This was intended to be a check on the idea that since the data appeared to be more nearly log-normally distributed, they would probably also yield better correlation coefficients on that basis.

The results of this summation for some of the results are given in table III.4. Those results not shown were very similar. The category giving the highest over-all correlations is the log-log category, on an ash-weight basis. To carry this check even further a manual search of the computer print-out was undertaken, and correlations above a certain arbitrary level noted. It was found that in all cases, the highest number of 'significant' correlations were found when a combination of log-log dry and ash-weight correlations was used because sometimes correlations was much better on one or other of the two bases. In only 9% of the cases were any correlations rejected on consideration of the log-log correlations that were significant in other categories, and in only 1% were the correlations in question not marginally significant anyway. I accordingly recommend screening for possible relationships on a log-log basis, and suggest the consideration of both ash and dry-weight bases.

Table III.4.

Comparison of bases of correlations; sums of correlation coefficients from various categories. (The correlations are from a wide range of elemental pairs.)

	Linear-linear		Log-linear		Log-log		Linear-log	
Correlation	Ash	Dry	Ash	Dry	Ash	Dry	Ash	Dry
W.racemosa wood-leaf	13.157	17.069	13.545	18.800	13.842	18.826	13.590	17.905
Q.acutifolia plant-soil	5.454	10.865	5.361	11.283	5.856	11.086	5.498	10.773
Q.acutifolia wood-soil	4.802	5.345	5.176	5.720	4.974	6.803	4.766	6.098

The only question remaining is the inevitable decision as to the level of probability which will enable a correlation to be confidently accepted as a real one. As already mentioned, this question has been made impossible, if a rigid dividing line is required, because the probabilities associated with correlation coefficients can only be considered even in a relative manner, in-so-far as the deviations from log-normality are of the same order. However, an arbitrary general region of acceptable probability was defined as follows.

If there were an array of 1000 correlation coefficients, all having the same probability, 0.001, then one of them would probably be spurious. From this example, a rule of thumb, of some use, is that for a given set of elements with associated identical probabilities, the probability of a single element of the set being spurious should not exceed the reciprocal of the number of elements of that set. For example if there were one hundred elements, the probability should not be less than 0.01, if ten thousand it should not be less than 0.0001 unless one was happy that a number of members of the group should be spurious.

In the present example, 82 members of the correlations calculated had associated probabilities of 0.005 or lower, which if they were all strictly comparable would mean there was once chance in six, that one member of the 82 was spurious. They are not strictly comparable, but this does afford a convenient screening device. In practice, those correlations having a probability up from 0.01 to 0.005 were also collected and put in a 'questionable' category. This actually may be rather too rigorous as is shown later for the uranium plant-soil relationships, but as a procedure it certainly led to the highlighting of interesting relationships.

(a) elements in soils

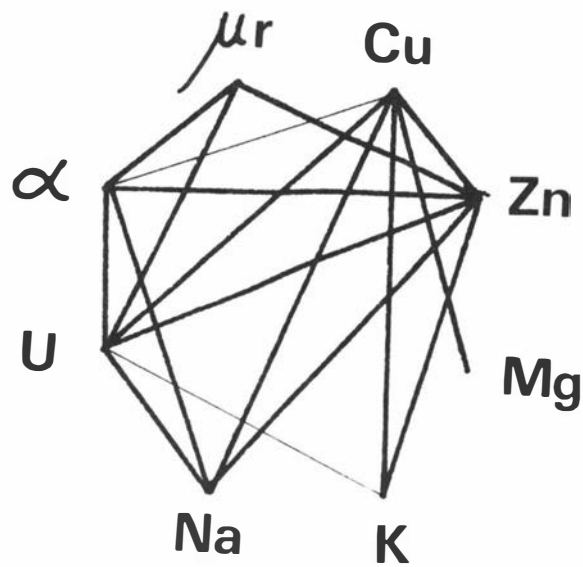
The correlations are illustrated in fig. III.6. There a large number of very good correlations, and magnesium and potassium seem to be the main elements that do not participate in this scheme. The correlations between uranium, the alpha counts and the microroentgens all have a very high probability associated with them (0.00000001), and those for the correlations between copper and uranium (0.0001) and zinc and uranium (0.0002) are also high. Some of these relationships are illustrated graphically in fig. III.7., which shows that alpha counts, copper and zinc can be usefully used as pathfinders for uranium in the soil. The finding that zinc is useful as a pathfinder is rather strange in view of the finding of Cohen et. al. (1969), that zinc was not related to uranium either in stream sediments, or the minerals themselves, and may be a sheerly fortuitous occurrence, useful only in this suite of soil samples.

Either the alpha counts or the scintillometer readings are good indicators of the amount of mineralisation in the soil, but the scintillometer readings are preferable, because they can, of course be done on the spot and do not require extensive sample preparation, and specialised and expensive counting equipment.

(b) elements in plants

(i) tissue distribution

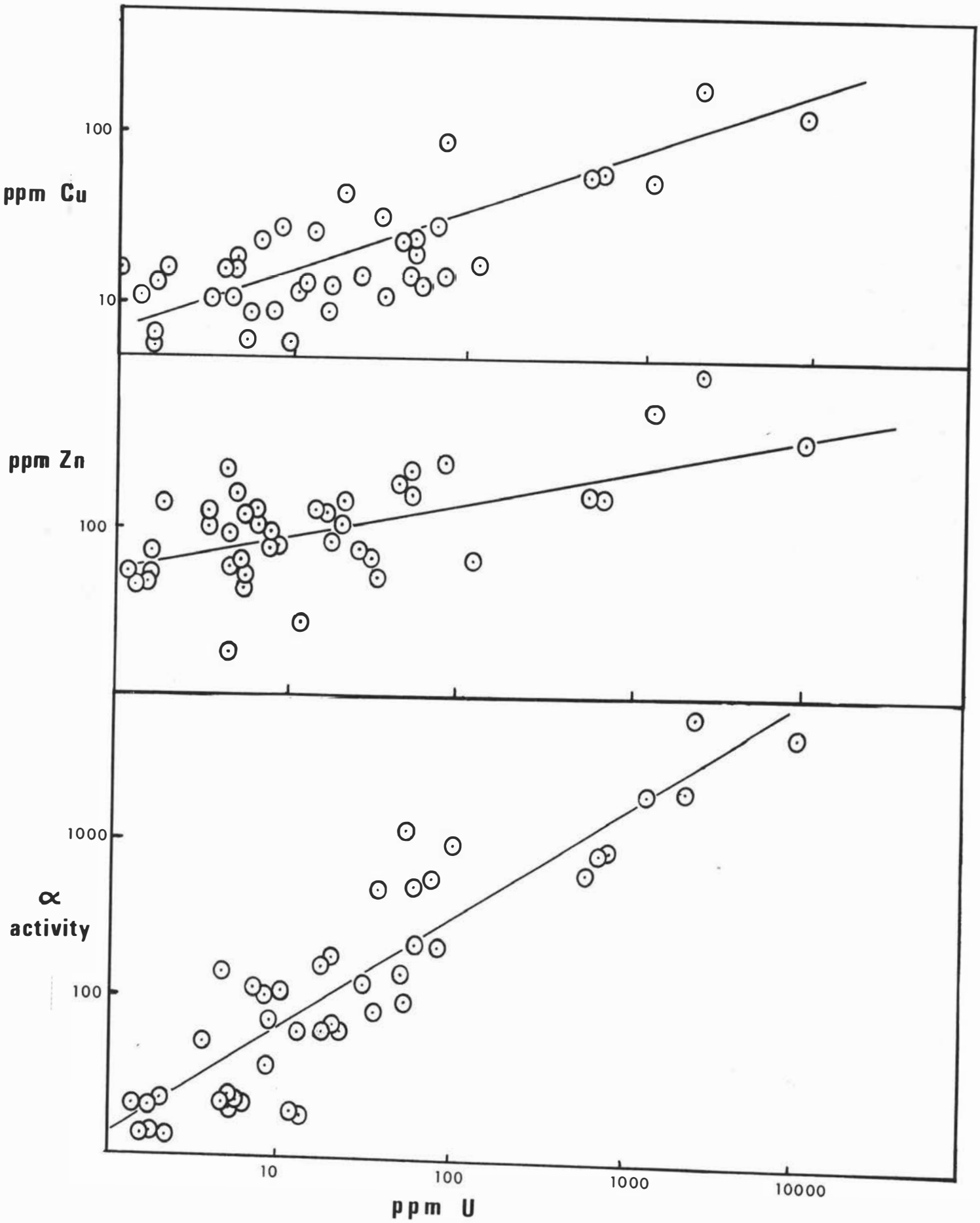
There was no correlation between the amount of any element in the twigs of a plant and the amount in the leaves. This points to the fact that in biogeochemical prospecting, the tissue chosen for sampling is of great importance. The most likely explanation for the lack of correlation is that the chemical form of the binding sites for various elements differs



0·01 – 0·005

< 0·005

probability level


100
10
ppm Cu
ppm Zn
100
1000
α
activity
100
10
100
1000
10000
ppm U

from tissue to tissue, and that the relative amounts of each also differ in a non-correlated fashion.

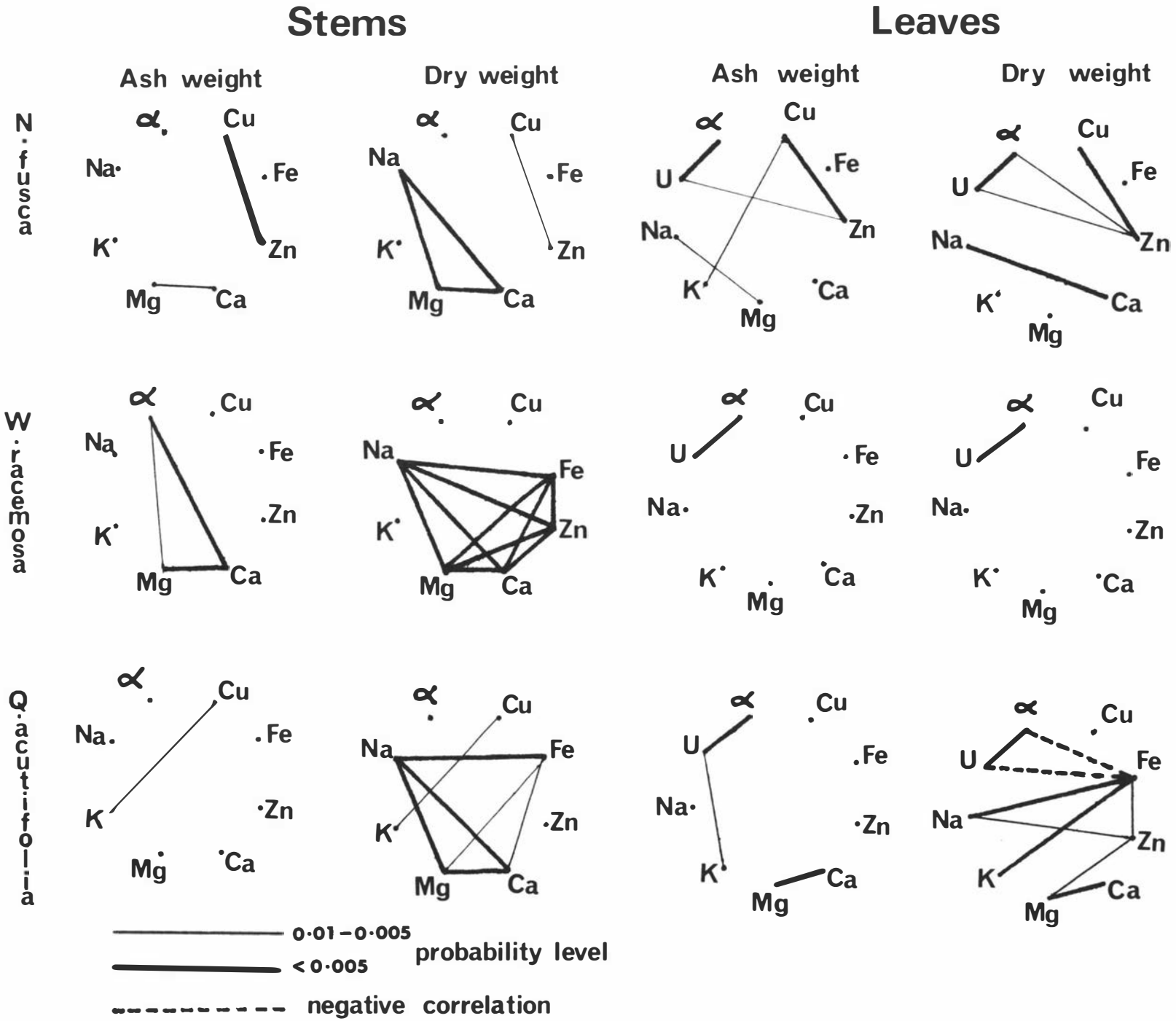
(ii) interelemental correlations

In contrast to the comparison of leaves with stems, many correlations were found for elements within a given tissue. Some of these correlations are illustrated in fig. III.8.; especially noteworthy are the large number of significant correlations for the twigs of W.racemosa.

In all cases there is a very good correlation between the alpha counts and uranium content of the leaf tissue, reaching a maximum probability of 0.00000265 for N.fusca leaves. These alpha counts are probably due to uranium itself r ther than to daughter products, especially radium, because in most cases the correlation of calcium with alpha counts was negative, and radium and calcium would be expected to behave similarly because of their chemical similarity. The negative correlation becomes highly significant for the twigs of W.racemosa on an ash-weight basis (with a probability of 0.000504).

There are good correlations between calcium and magnesium, and in a few cases, between copper and zinc. These relationships are well known (Walker, 1954; Walker et. al., 1955; Warren and Delavault, 1949), and have been used as indicators of mineralisation, because on mineralised ground, the usually constant ratios, are disturbed. Here, the very fact of good correlation, suggests that the disturbance of the ratio is minimal, but this is evaluated more precisely shortly.

Unfortunately there are few correlations of elements with either

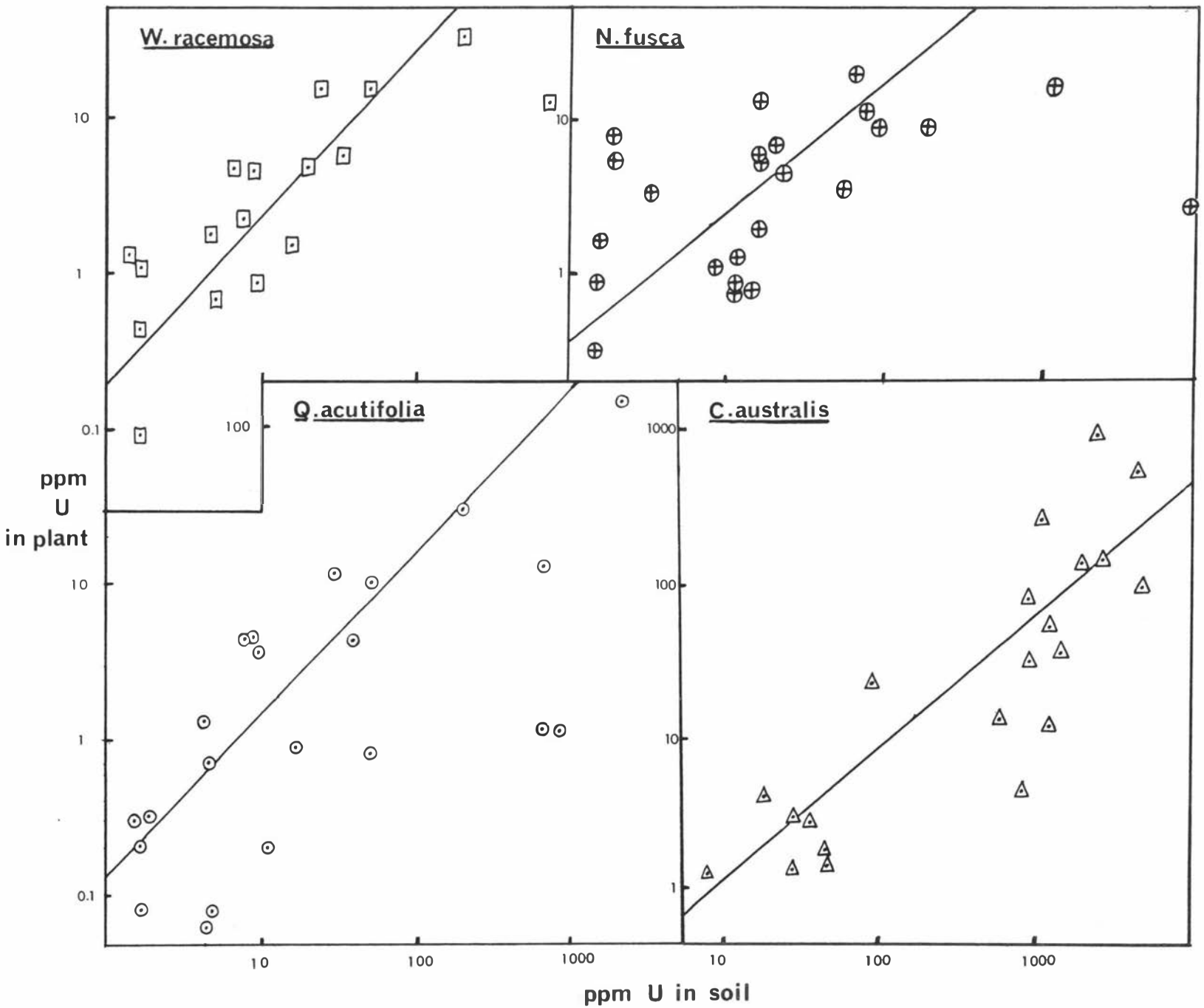


the alpha counts or the uranium content which might potentially be useful for indirect indication of the amount of either in the soil. However, for Q.acutifolia, iron correlates negatively with both the uranium and alpha counts (with probabilities of 0.0006859, and 0.0002356 respectively). There is a possible physiological reason for this, which is discussed in subsection (c). For N.fusca, there may be a weak correlation of uranium with zinc. The actual value of this as an indirect indicator of the amount of uranium in the soil is also discussed in the next subsection.

(c) plant-soil correlations for various elements

Since so many correlations were calculated, it was possible to determine whether elements in the plants bore any relation to the uranium content of the soil, and, at the same time, to determine whether any elements in the plants bore a relation to the amount of other elements in the soil, such as copper and zinc.

It is possible that the level of significance adopted was too high, for one of the findings of this section was that except for the Quintinia the uranium plant-soil correlations were only marginally significant. However, as shown in fig. III.9. for four species, a relationship exists, and the species are probably useful for biogeochemical prospecting. The corresponding graphs for the alpha counts are better (fig. III.10.) and the relationships are included in the table (table III.5) of those found to be significant. Also, an examination of the normality statistics shows that the uranium figures depart furtherest from normality of any set of data, in that they are more spread out, which probably means the correlation coefficient was not such a good measure of the true association that exists. There was no relationship between the pH of the soil and the uranium plant/soil ratio (the


W. racemosa
N. fusca
Q. acutifolia
C. australis
ppm U in plant
ppm U in soil
0.1
1
10
100
1000

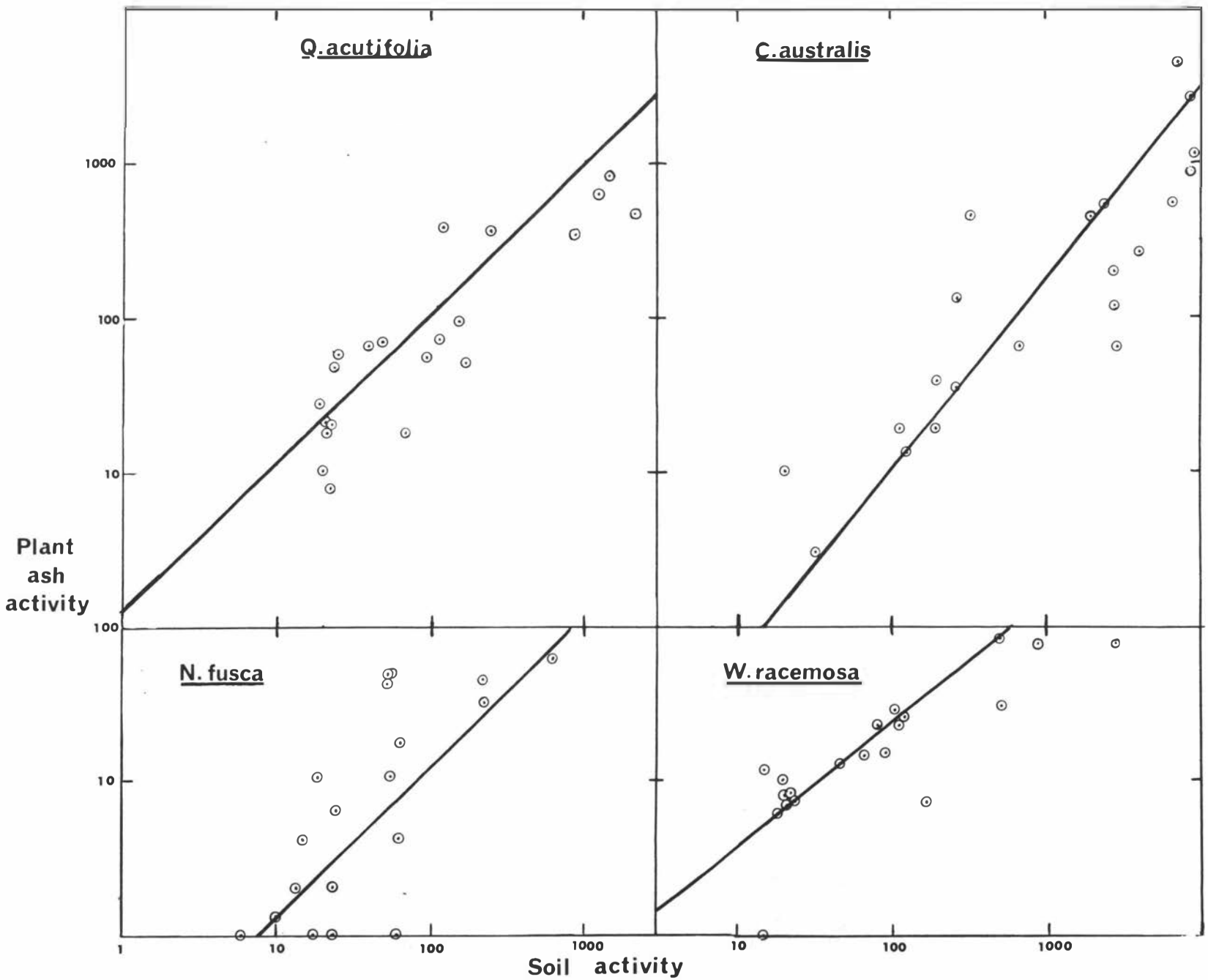

Q.acutifolia
C.australis
N. fusca
W.racemosa
Plant ash activity
Soil activity
1000
100
10
1
10
100
1000

Table III.5.

Levels of significance of plant-soil correlations

Plant	Part of plant	Correlation basis	Element	Significance level
N.fusca	twigs	ash weight	K	0.0000275
"	leaves	" "	α	0.00183
"	"	dry weight	"	0.00231
Q.acutifolia	twigs	ash weight	"	0.00000487
"	"	dry weight	"	0.0000119
"	leaves	ash weight	"	0.00000786
"	"	" "	U	0.0000648
"	"	dry weight	α	0.0000784
"	"	" "	U	0.000107

probability being 0.5).

An examination of the contents of table III.6., shows that there is a high negative correlation between iron in W.racemosa twigs, and to a lesser extent iron in Q.acutifolia leaves, and the amount of uranium in the soil, enabling the iron content to be used as an indirect indicator of mineralisation. This appears to be the first time iron has been suggested for this role in uranium prospecting, although the idea is known for other elements. The idea may be of even more general applicability, for there were also negative correlations (not significant) for N.fusca though with a small magnitude. According to a review by DeKock (1964) some metals compete with iron for entry into the plant, and this could cause a reciprocal relation to arise within the tissues of the plant itself, and also cause disturbances in the potassium/calcium ratio, an idea investigated in the next subsection. Since a deficit of iron causes chlorosis, the sickly appearance of the leaves in Plate III.6 is not surprising, but this is not useful as a prospecting guide because it only occurred in extreme conditions (i.e. very high uranium content).

Table III.6 shows other correlations, but the only ones of immediate practical interest are the correlation of the potassium levels in ashed N.fusca stems with the amounts of copper and zinc in the soil. The other correlations are of less general interest, involving as they do the alpha counts and uranium levels, which will not apply to other areas lacking uranium mineralisation.

(d) elemental ratios as indicators of the soil uranium content

The copper/zinc ratio, the calcium/magnesium ratio, and the

Table III.6.

Correlations of pathfinders in plants with soil constituents.

Plant	Part of plant	Correlation basis	Element in plant	Element in soil	Significance level
N.fusca	twigs	ash weight	K	Cu	0.00338
"	"	" "	"	Zn	0.00808
"	leaves	" "	U	Zn	0.000328
"	"	" "	∝	"	0.00499
"	"	" "	"	K	0.00335
"	"	" "	"	U	0.00118
"	"	dry weight	U	Zn	0.000434
"	"	" "	∝	"	0.00201
"	"	" "	"	K	0.00501
"	"	" "	"	U	0.00124
W.racemosa	twigs	ash weight	Fe	Na	0.000534*
"	"	" "	"	U	0.00960*
"	"	" "	∝	U	0.00159
"	"	dry weight	Fe	Na	0.00155*
"	"	" "	∝	U	0.00871
"	leaves	ash weight	U	∝	0.0000265
"	"	dry weight	"	"	0.0000595
Q.acutifolia	twigs	ash weight	∝	Zn	0.000685
"	"	" "	"	Na	0.00175
"	"	" "	"	K	0.00420
"	"	" "	"	U	0.00174
"	"	dry weight	"	Zn	0.00135
"	"	" "	"	Na	0.00240
"	"	" "	"	K	0.00492
"	"	" "	"	U	0.00283
"	leaves	ash weight	U	∝	0.0000889
"	"	" "	∝	U	0.00235
"	"	dry weight	Fe	"	0.00671*
"	"	" "	"	∝	0.00594
"	"	" "	U	Zn	0.00802
"	"	" "	"	∝	0.000236
"	"	" "	∝	U	0.00503

*Negative correlation

potassium/calcium ratio were all correlated against the soil uranium content, but none of the correlations was even remotely significant, and we conclude these ratios are of no value for biogeochemical prospecting.

D. Discussion.

The above work shows that analysis of plant material can indicate the levels of an element likely to be found in the soil, though not all species may be equally good, since the uranium levels in Carpodetus serratus appeared to bear no relation to those in the soil.

Most of the levels of uranium found in the plants were surprisingly high, which may be a function of the persistent dampness of the area, and constant movement of uranium in the ground water to cover a large area and give a large halo of uranium around the ore body. The figures from this preliminary survey suggest that a value of uranium greater than about 1-2 ppm in plant tissue suggests favourable ground for further exploration but the values must be much higher to represent actual mineralisation.

Other parameters also correlate with the amount of uranium in the soil, one of these being the amount of iron in the plant (which negatively correlates with uranium in the soil), and of course, the alpha counts, which require much less effort to obtain than results of uranium analyses. The alpha counting is automatic, other work may be done at the same time, and from the graphs the indication of alpha emission of soil is rather better than for the corresponding uranium graphs. Various correlations found in this section indicate that the alpha count rate is very probably due to uranium and little else, so this technique is fairly reliable. The high correlation of uranium and alpha counts does not prove one is derived from the other of course, and this problem is explored in section V.

Plant samples are about three times quicker to collect than soils under the conditions described but the soils presumably reflect the nearby

mineralisation rather more accurately. One simple solution to this conflict is the use of the scintillometer, since it was shown that there was an excellent relationship between the reading from this instrument and the uranium content of the soil, and no analysis is required.

As a further question, it might be asked, why use a scintillometer on foot? Surely aerial surveys are cheaper and faster? This is normally true, but under the very rough conditions in the Buller Gorge, nearly half the radioactive anomalies found from the air by helicopter proved on ground follow-up to be due to the 'mass effect' or the result of nearby cliffs or large land masses increasing the count-rate artificially (Whitehead and Brooks, 1968). Under New Zealand conditions, ground follow-up will usually be needed, and the methods do not supersede one another.

The advantages and disadvantages of the various methods used in the area are summed up in table III.7. One of the advantages of biogeochemical techniques is that they can potentially detect buried deposits, and it is conceivable that this advantage (admittedly of rather limited value in an area with a rainfall as high as that of the Buller Gorge) could be combined with that of aerial scintillometry. Balabanov and Kovalevskii (1963) have described how it is possible, even with conventional equipment, to detect actual biogeochemical anomalies, that is, the high levels of uranium in the actual leaves, many feet above the ground. In view of the difficult travel in the region this idea is rather appealing, but is limited ultimately by its sensitivity, which cannot be as good as that of a laboratory analysis. Since fusion reactors are unlikely to be in use before the turn of the century, and the world demand for power is expected to multiply 8-10 times by then, the finding of even low-grade uranium ore deposits will increasingly

Table III.7.

A comparison of methods of prospecting for uranium

Material Studied	Soils				Vegetation			Stream Sediments	Stream Waters	General Terrain
General Comments on Material	Easily collected except in thickly-forested areas. Do not necessarily show presence of uranium if non-mineralised overburden exists. More difficult to analyse than vegetation if samples have to be dissolved.				Preliminary orientation survey is needed before biogeochemical method can be used. Usually less reliable than soil for indicating the presence of uranium. Advantage of ease of sampling and ease of analysis. Can sometimes indicate the presence of uranium beneath a non-mineralised overburden.			Usually not reliable due to ease of leaching of uranium. Can be effective in proximity of an anomaly.	Unreliable when flow rates vary. Anomaly should form appreciable fraction of watershed.	
Method Used	α-activity	β-activity	γ-scintillometry	Fluorimetry	α-activity	β-activity	Fluorimetry	Fluorimetry	Fluorimetry	Aerial γ-Scintillometry
Advantages	Relatively speedy and economic of operator's time.	Relatively speedy and economic of operator's time. More sensitive than α-count.	In situ measurements speediest of all.	Most reliable method to give uranium concentration.	As for soils.	As for soils.	As for soils.	As for soils.	As for soils.	Unsurpassed for speed and cheapness per unit measurement.
Disadvantages	Does not always indicate uranium.	Unreliable at low activities due to ^{40}K. Does not indicate uranium.	Does not indicate uranium. Only indicates daughters such as Ra.	Slower than α-count in total operator time but quicker for overall speed of analysis.	As for soils.	Very unreliable at low activities due to large amount of ^{40}K in plants.	Speedier analysis than for soils.	As for soils.	Reliability impaired by fact that amounts of uranium are near limit of method.	Can be misleading due to mass effect. Always needs ground follow-up.
Overall rating of method	Excellent	Poor	Fair	Excellent	Good	Poor	Good	Poor	Fair	Excellent

be important for the construction of fission reactors, and the utmost sensitivity in prospecting will be necessary. Biogeochemical analysis is thus still important. One development which should be of great use in New Zealand, is the current design and development of a foliage reaper for use suspended beneath a helicopter, which, although primarily required by the Forestry Service, could also be used in biogeochemical prospecting from the air, and would be much faster than conventional techniques.

It can thus be seen, that tall forest species like N.fusca, and W.racemosa which are now known to reflect the amount of uranium in the soil, could be used in a programme of biogeochemical prospecting at some future date in New Zealand.

SECTION IV

AQUATIC BRYOPHYTES AS INDICATORS OF MINERALISATION

A. Introduction

One of the striking features of the work described in the last section, was the very high amount of uranium found in the lichen and moss gathered from near the area of high mineralisation. A survey of the literature showed that in general, lower plants have a tendency to concentrate large amounts of different cations.

Studies by Lounamaa (1956) and LeRoy and Koksoy (1962) have shown that lichens may accumulate such unusual elements as gallium, and the rare earths. Chromium and lead are remarkably high, though this varies with the type of rock substrate.

The accumulation of elements by mosses has been studied extensively, and it is well known (Shacklette, 1967) that the genera **Merceya** and Mielichoferia are resistant to, and may even prefer, a substrate with excessive amounts of copper. Chapman and Shacklette (1960) have also described a bryophyte that may be an indicator of the presence of lead. Ono et. al. described specimens of Parmelia tinctorum and Vittaria frexuosa containing 14,580 ppm Zn and 18,600 ppm manganese respectively, and Samoilova (1961) found that Scorpidium scorpoides and Calliargon giganteum accumulate uranium.

Other moses and lichens absorb large amounts of natural radioactivity (Grodzinskii, 1959; Penna-Franca and Gomes de Freitas, 1963; Marsden, 1964). Lead and polonium from fall-out are absorbed so efficiently that the resultant high levels can present a hazard to man via a food chain involving the reindeer, as reported by Chandler and Wieder (1963), Beasley and Palmer (1966), Bovard and Grauby (1967) and Kauranen and Miettinen (1967).

The latter authors found about 6 pCi/g of activity in Finnish lichens compared with about 0.3 pCi/g for other plants examined. Chandler and Wieder (1963) suggested that one possible reason for this extensive accumulation might be the relative longevity of mosses and lichens compared with annual species of vegetation.

Accumulation is not confined to terrestrial forms, as Webb and Fearon (1937) and Webb (1937) showed that marine algae accumulate barium, while Kunasheva (1944) and Wiesner (1938) repeated the finding for radium. Some of the stranger findings were one species of thermophilic algae (Umemoto and Mifune, 1953) which contained large amounts of germanium, and another observed by Hoffman (1941) which contained uranium. Uranium has since been observed in other algae, (Koval'skii and Vorotnitskaya, 1965; Koval'skii and Vorotnitskaya, 1966; Koval'skii et al., 1967) and in seaweeds (Zlobin, 1966).

Since accumulation is very marked in many cases, it might be imagined that lower plants are ideal for biogeochemical prospecting, but their root systems or rhizoids, are so small that they can only sample a very small volume of rock or soil. This can be useful if the ore-body outcrops, but if that happens, other prospecting techniques are also applicable. However, Persson (1948) has described a technique of prospecting involving studying the collection localities of cuprophilic mosses and lichens in herbaria and then applying conventional methods of prospecting to these areas.

There is another method of prospecting, stream-water sampling, which suffers from precisely the opposite defects. An ore body may be deeply buried, and have no outcrops. If it is very deep underground it may not be possible to apply biogeochemical techniques, or even geophysical techniques.

However, if ground water is flowing through it, then minute amounts of the mineral may be leached out, and may eventually arrive at the surface to be further transported by streams. Provided sufficiently sensitive analytical techniques are available, it may be possible to detect the slightly elevated concentration levels of the element sought. It may sometimes be easier to use as a pathfinder, a more abundant element, or one for which the analysis is more sensitive. Many workers in most countries have attempted stream-water sampling for uranium (e.g. Fix, 1955; Saukov, 1955; Lecoq et al. 1958; Wodzicki, 1959b; Peacock, 1961; Jurain, 1962a; Leutwein and Weise, 1962; Deszi, 1963; Tilak and Aswathanarayana, 1963; Chamberlain, 1964; Miyake et al., 1964), but a major disadvantage is that the amounts normally present in stream water may border on the limits of sensitivity. Another problem is the generally neglected possibility of uranium adsorbing on to the walls of the container from such very dilute solutions. Koczy (1950) and Robertson (1968), have shown that even polythene may adsorb a substantial amount of uranium over the course of a few weeks (in the former case 75% of uranium in seawater was adsorbed on to glass in three weeks). The adsorption will depend on the previous history of the container, the pH of the solution, and the concentrations of other cations competing for adsorption sites.

To avoid transporting large volumes of liquid from the field, it is possible to use an apparatus similar to that devised by Roslyakov and Ezhova (1966) which passes twenty litres of water through a bed of activated charcoal to absorb the uranium, following which the charcoal is returned to the laboratory for analysis, but under New Zealand conditions, another disadvantage still remains, in the form of the high and variable rainfall, which causes wide fluctuation in the uranium levels of stream

waters (Wodzicki, 1959b). It occured to me that the disadvantages of moss analysis and stream water sampling might conceivably be removed by combining both methods.

If bryophytes were sampled from a stream, the stream waters themselves, flowing from considerable distances, and partially underground, would act as a kind of extended root system for the bryophytes, and the immense concentrating ability, and longevity of the mosses, would counteract the low concentration, and variability of the elements in the streamwaters themselves. The method is thus potentially superior to more normal biogeochemical techniques. It seemed it would be specially applicable in New Zealand, which has one of the richest moss floras in the world. Further searching of the literature revealed some parallel ideas, but these had not been directly related to prospecting. Fall-out monitoring had been the usual application (Beninson et al., 1964; Garder and Skulberg, 1966; Ravera, 1966).

The nearest approach to the ideas outlined about is the use of peat bogs for prospecting. Rather than detection of deposits up stream, however, the investigations are usually concerned with determination of the type of ground underneath the bog, and Salmi (1955) claimed that deposits could be detected as deep as forty feet, through seepage of minerals to the surface and chelation by the bryophytic material.

Eckel et al., (1949) found copper-bearing peats downstream from a known copper deposit, and Cannon (1955) and Salmi (1955, 1956) found large amounts of zinc and lead in such places. There are similar reports of uraniferous bogs (Debnam, 1955; Bowes et al., 1957; Moiseenko, 1959; Armands, 1961; Armands and Landergren, 1960; Lisitsin et al., 1967), and

the amounts present may be high enough to warrant exploitation. Unfortunately the problems of extraction have not been completely solved, so at present such bogs act mainly as indirect indicators of adjacent mineralisation or high background levels.

It is not certain how the uranium is chelated on to the decaying moss and peat material, but some authors have shown that it may be bound by the carboxyl groups to the humic acids and melanoidines (Manskaya et al., 1956; Szalay, 1957).

To investigate the feasibility of using individual bryophytes for prospecting, samples of moss material were collected from various streams draining mineralised areas in the Buller Gorge, and analysed for uranium, and elements known from previous work to be pathfinders for this metal. (Cohen et al., 1969). Parallel experiments were also undertaken in which homogeneous peat material was placed into cloth bags and allowed to absorb uranium from the stream water for seven days. The purpose of this was to obtain an independent value for the uranium content of the streams, not influenced by differences of absorption due to species differences among the mosses. This section of the thesis describes the results of the investigations and attempts to evaluate the new techniques.

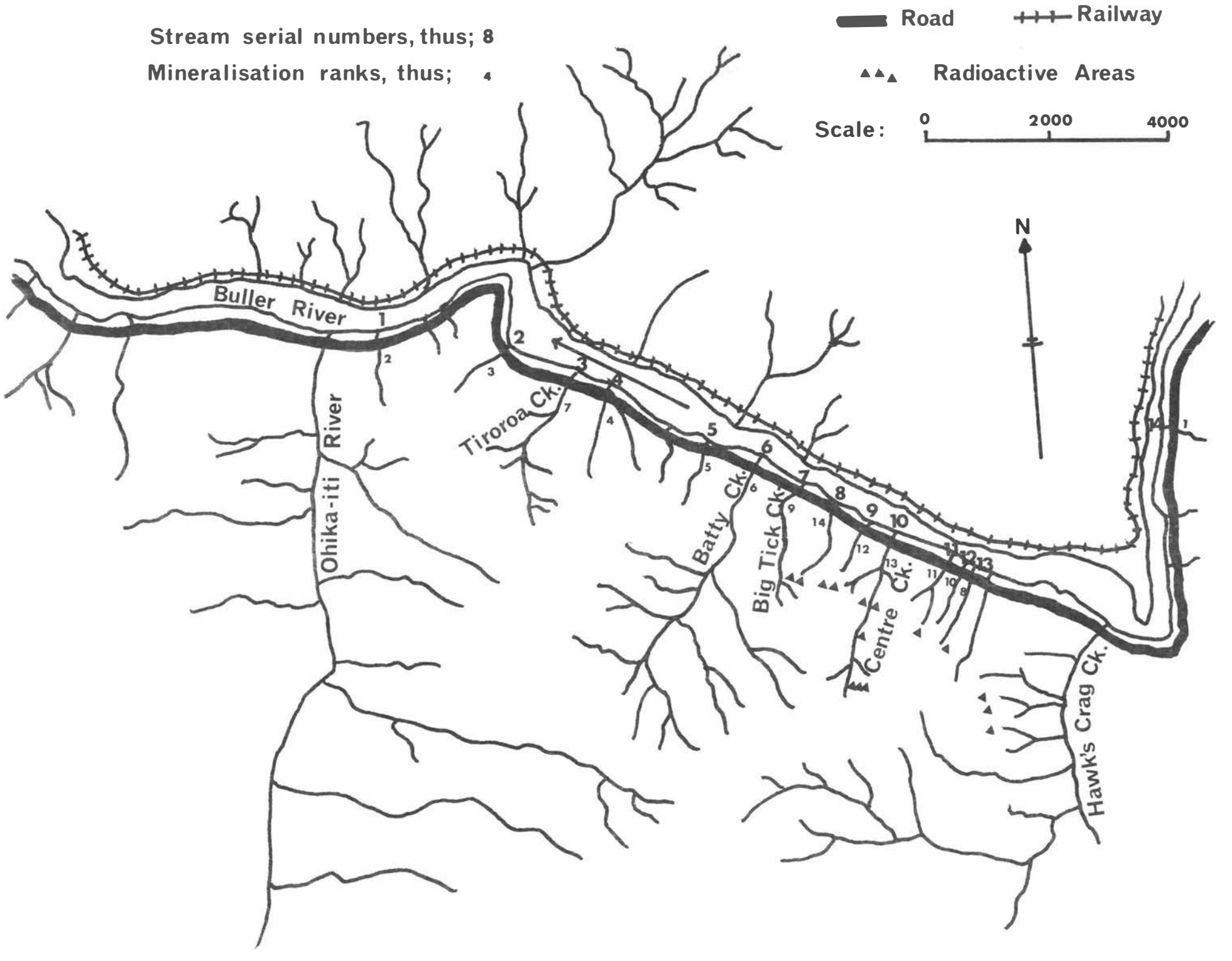
B. Methods

1. Fieldwork

Approximately six bryophytes were collected from each of the streams shown in fig. IV.1. A specimen of the rocky substrates was also collected for analysis.

The bryophytes identified were Bryum blandum (H.f.&W.), Dicranella vaginata (Hook) Card., Distichophyllum pulchellum (H.f.&W.) Mittl, Fissidens rigidulus C.M., Lophocolea planiscula (Tayl.) G.L.&N., Plagiochila deltoides Lindenb., Pterogophyllum dentatum (H.f.&.W.) Mitt., Riccardia sp. and Thamnium pandum (H.f.&W.) Jaeg. Some of these are shown on plates IV.1. and IV.2. The specimens depicted are particularly high quality, and often in the field it was found that mosses growing in very swift-flowing streams had been very much worn by the water, rendering identification less easy. Identification was also made difficult by the occurrence of the mosses in composite clumps, the amount of any one moss often being too small to analyse. Another problem was the basic similarity in appearance of many of the mosses. If the technique was to be of any practical use, indication of uranium would have to be independent of species differences, so this factor was henceforward neglected and in all subsequent work, composite samples of mosses were collected. Another factor influencing this decision was the finding that not all mosses occurred in every creek, the total number of specimens of any given species being too low for independent evaluation.

In a separate attempt to sample stream waters by bryophytic material, but eliminating species differences, New Zealand Hauraki peat was washed successively in tap water, distilled water, ethyl alcohol and benzene as recommended by Horvath (1960) and was dried at 110^{0}C. Ten-gram samples


Stream serial numbers, thus; 8
Mineralisation ranks, thus; 4
Road
Railway
Radioactive Areas
Scale: 0 2000 4000
N
Buller River
Ohika-iti River
Tiroroa Ck.
Batty Ck.
Big Tick Ck.
Centre Ck.
Hawk's Crag Ck.

of this material were sealed in finely-woven cotton bags, and were anchored in stream beds for a period of seven days, which is the time shown to be necessary for equilibrium (Horvath, 1960). As shown in fig. IV.1. the total number of streams was fourteen. The flow rate of each stream was recorded.

2. Analysis

When the moss material was washed in tap water and distilled water, a large amount of fine silt collected on the bottom of the washing vessel. This sediment had been trapped in the rhizoids of the plants, and this property is made use of by local prospectors when sampling stream sediments in regions where little sediment would normally accumulate in the stream bed itself (personal communication, J. Braithwaite, 1969). The sediments from the mosses were individually collected and analysed, since it seemed possible that some sediment might not have been washed out of the rhizoids, and the final analysis would be erroneous. There was also the remote possibility of the mosses themselves absorbing some part of their cation content from the sediment and rock instead of the stream water. Comparison of the sediment analysis with the bryophyte analysis would show if either of these factors was important.

The treatment of the mosses then followed the procedure described in section I for plant material. Uranium was determined by fluorimetry, alpha counts by the Low-beta II instrument, and lead, copper and beryllium by emission spectrography.

C. Results

1. Evaluation of the possibility of contamination

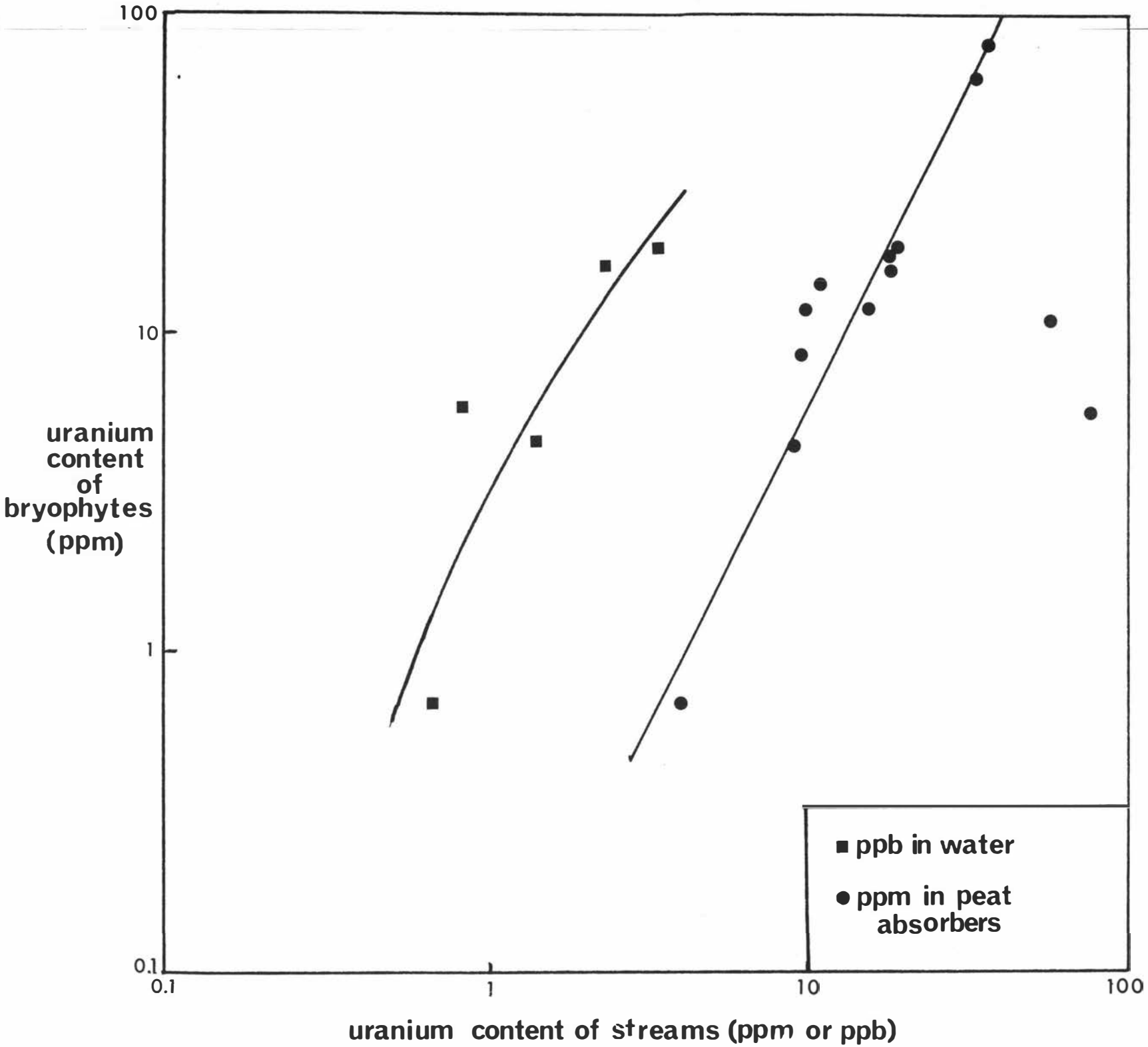
(a) ash fraction

According to Shacklette (1967) if the percentage ash of a bryophyte exceeds 7-10%, contamination should be suspected. This will, of course, vary with different mosses, and in the same paper the average ash value for the genus Mielichoferia is given as 15%. The mean of the ash percentages from the Buller Gorge bryophytes was 16% which probably indicated that the degree of contamination was low, and was of the order of 0-6%.

(b) elemental content of silt and rocky substrate

Mean values for elemental concentrations in sediments (host rocks were even lower) were: beryllium, 3.1 ppm; copper, 63 ppm; lead, 54 ppm; uranium, 5.8 ppm. Comparison with the data shown in Table IV-3 indicates that contamination is likely to be negligible, except possibly in the case of copper.

The probability level of a correlation between the bryophyte uranium content and the sediment uranium content (on a logarithmic basis) was 0.074, and for the bryophyte uranium content and the rock substrate was 0.173. From the criteria in section III neither of these is a significant correlation, which suggests that the bryophyte uranium content was derived from neither sediment entrapped in its rhizoids nor from the base rock to which those rhizoids were attached. That the elements in the bryophytes were derived from the stream water is shown rather more directly in fig. IV.2., where a correspondence between the stream water content and the bryophyte content is apparent.


100
10
1
0.1
0.1
1
10
100
uranium content of bryophytes (ppm)
uranium content of streams (ppm or ppb)
ppb in water
ppm in peat absorbers

The uranium in the peat is most likely derived from the water since contamination in this case is highly unlikely and fig. IV.2 also shows that the moss uranium contents are related to the peat uranium contents, which again, though indirectly, suggests that little contamination occurs for uranium, and that the elemental content of the bryophytes is derived from material absorbed from the streams.

2. The elemental content of bryophytes

The following analysis figures are in terms of an ash weight basis, unless other-wise stated.

(a) lead

The range of lead values in the mosses was 45 - 1400 ppm, with mean 340 ppm. The range of results quoted by Lounamaa (1956) is greater, and many non-aquatic bryophytes have lead levels as low as 10 ppm. The mean of the results reported by Shacklette (1967) is comparable, but his previous figures (Shacklette, 1965) had a mean of 1860 ppm even on a dry-weight basis.

(b) copper

The mean value was 40 ppm compared with 300 ppm in the specimens studied by Lounamaa (1956), and 2000 ppm (approximately, on an ash weight basis) for the specimens studied by Shacklette (1965). It seems there is exceptionally wide variation in the amounts of this element found in mosses, the more so since the 'copper mosses' contain about 10,000 ppm on an ash weight basis (Shacklette, 1967).

(c) beryllium

Meehan and Smythe (1967) have shown that the amount of beryllium

in acacia ranges from 0.1 to 1.0 ppm and most other vegetation has even less. Mosses are exceptional, since Shacklette (1965) showed that his specimens contained an average of 6 ppm even on a dry weight basis. On an ash weight basis this is equivalent to about 60 ppm, which is similar to the mean of 50 ppm found in the present study. The lowest and highest figures were 4.2 and 600 ppm respectively. The presence of such high levels of such a potent enzyme inhibitor in an apparently healthy tissue, poses grave biochemical problems.

(d) uranium

The range was 0.7 - 160 ppm with a mean of 19 ppm. I was unable to obtain comparable figures from elsewhere.

(e) alpha counts

These resembled closely the alpha counts obtained from higher plants growing in a mineralised area. The values were mostly 20 - 50 counts per 100 minutes per 50 mg ash. This is in sharp contrast to vegetation growing in soil near the mosses, which gave zero or one alpha count in the same period.

The ash of the samples contained from 4 - 324 ppm uranium with a mean of 15 ppm if the highest point is rejected. This latter figure was caused by contemporary dumping of uranium-rich spoil into the head of the stream, from exploratory mining operations. Fortunately this affected only the peat results, which were a later experiment than the moss collection. Apart from this one aberrant figure, the mean of the uranium contents of the peat bags, is similar to that of the mosses.

3. Statistical correlations

On analysis by the computer programme described previously, most of the data (even on a logarithmic basis) were not normally distributed, but positively skewed.

(a) uranium and alpha counts

Rather surprisingly, there was, on a linear basis only, a negative correlation ($P = 0.01$) between alpha counts and uranium content in the mosses. The value of this probability indicates that there is only a possibly significant relationship, but if it indeed exists, there is an interesting possible reason for it.

It is shown later in this thesis, that when examined by gamma-ray spectroscopy, one of the commonest bryophytes contained substantial quantities of radium when taken from a mineralised stream but none when taken from an unmineralised stream. For a mineralised stream more than fifty per cent of the alpha counts are due to radium and daughters, and if this is typical, then it is possible there is competition for the same binding sites on the moss, with radium preferentially displacing uranium. A moss with a low uranium content could therefore have a high alpha count, which means that alpha counts of moss material are of no use for prospecting purposes.

(b) local mineralisation, and the elemental concentration of bryophytes

The probability values for some of the interelemental correlations for the bryophytes are as in table IV.1. The dry-weight correlations are much superior to the ash-weight correlations, which means

Table IV.1

Significance levels of interelemental correlations for bryophytes

	Correlation					
	Pb v. Cu	Pb v. Be	Pb v. U	Cu v. Be	Cu v. U	Be v. U
Dry-weight basis	0.000066	0.00000139	0.000301	0.00000003	0.116	0.00185
Ash weight basis	0.202	0.0114	0.0139	0.397	0.151	0.073

that the organic part of the mosses is probably more important in determining the elemental content than the inorganic matter.

There is a strong relationship betwcen several pairs of elements in the bryophytes, and the relationship between copper and beryllium is especially strong. The reason for the correlations is probably that there are different binding sites for different elements, and that these exist in similar proportions in most mosses. Negative correlations would have indicated competition for the same sites. The lack of correlation for the uranium figures suggests that the chelation sites of uranium are not present to the same extent in different mosses.

It was a much more difficult matter to evaluate whether the method is useful for prospecting. It is possible that there are substantial underground deposits that are detected by analysis of the mosses, but are otherwise unknown at present. There is no allowance that can be made for this, and the method was hence judged solely on the basis of known mineralisation.

From the known mineralisation of each catchment area, a rank was assigned to the appropriate stream, ranging from 14 for the most mineralised stream, to 1 for the least mineralised. Eight of the fourteen streams contained mineralisation, but the mineralisation was weak in two cases. The ranks assigned are shown in fig. IV.1.

The ranks obtained were correlated against the following elemental contents in the mosses: lead, copper, beryllium, uranium, uranium divided by flow rate, uranium divided by watershed area, and uranium divided

by both catchment area and flow rate. The catchment area (determined from aerial maps) and flow rate were taken into account, because of the possibility that a mineralised area was only producing a very small effect in the moss concentration because of dilution by the stream water. The probabilities for the various correlations being spurious are shown in table IV.2.

The results suggest that there is a relation between the mineralisation ranking and the uranium content, but not between the rank and the other elements present in the mosses. The relationship between the rank and the uranium/area ratio is shown in fig. IV.3. It is possible that if a more systematic way of ranking the streams had been found, the result would have been slightly better, but there is an inherent limitation from the rather large spread of the bryophyte elemental contents, for each stream.

4. Use of games theory for evaluation

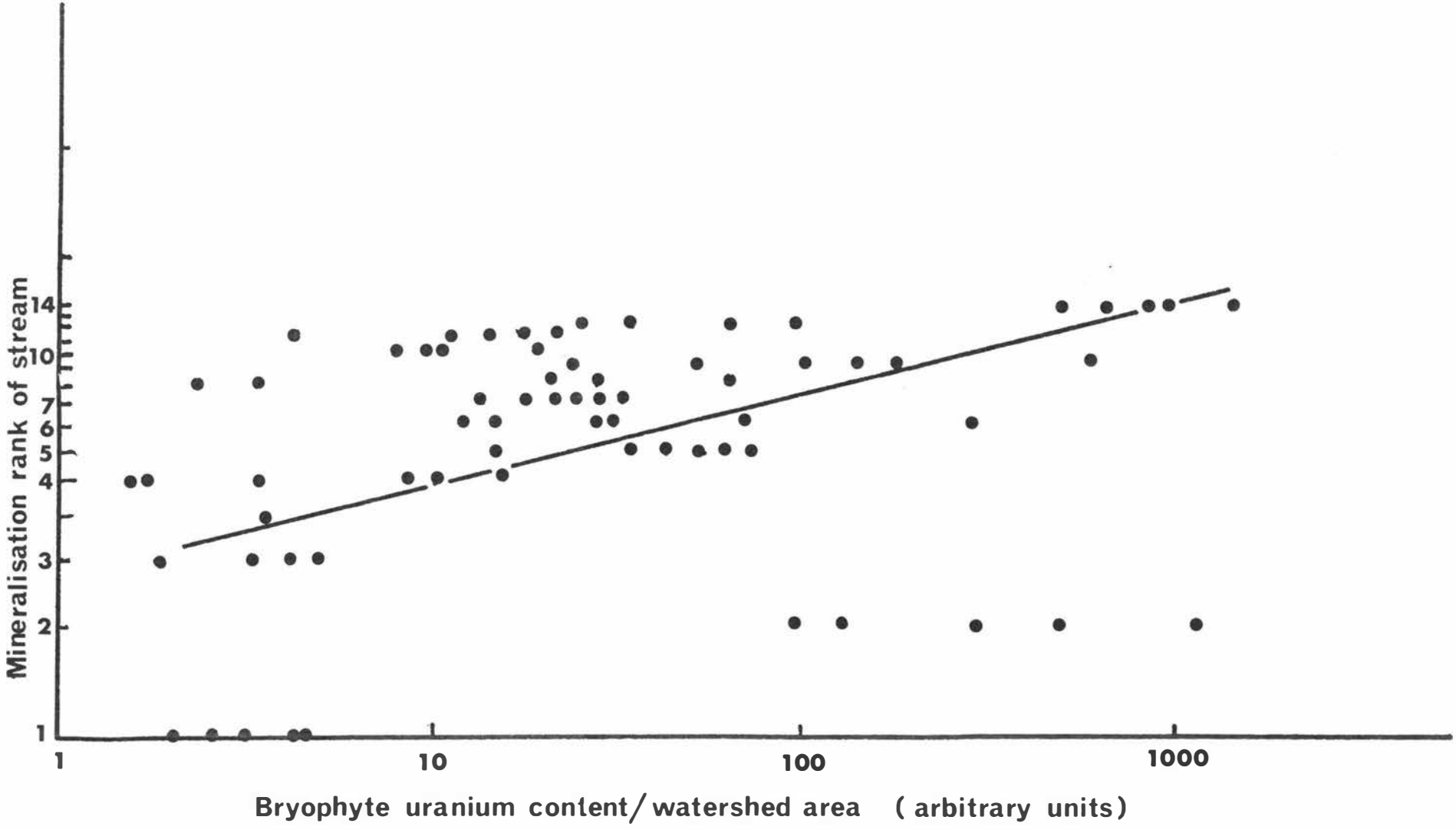
There is another possible way of evaluating the use of bryophytes for prospecting. This is based on a very simple use of games theory (Von Neumann and Morgenstein, 1953).

The amount of an element in a bryophyte may indicate that the stream drains mineralised ground, or, if very low may indicate that no mineralisation is present. Either of these indications may be correct or incorrect. For example, it is possible that for some reason a bryophyte in a mineralised stream has a very low content of either uranium or some other pathfinder, or that, through some extraordinary concentrating mechanism, a moss in an un-mineralised stream has a high amount of uranium. It is possible to assess how well these indications measure up to reality, if we have a situation, as in the present example, where the mineralisation near

Table IV. 2

Significance levels of correlations between mineralisation rank and bryophyte elemental content

	Correlation of mineralisation rank with						
	Pb	Cu	Be	U	U/flow rate	U/area	U/(flow rate x area)
Ash weight basis	0.209	0.019	0.453	0.0050	0.00000175	0.00000023	0.000000425
Dry weight basis	0.296	0.01	0.499	0.00079	0.00000087	0.00000013	


Mineralisation rank of stream
14
10
7
6
5
4
3
2
1
1
10
100
1000
Bryophyte uranium content/ watershed area (arbitrary units)

all streams is known. We could, for example, record the number of predictions, and see how many correct predictions were made, on the basis of examining uranium content, copper content, and so on. The final scheme adopted was slightly more complex than this, partly due to the presence of weak and doubtful mineralisation in some streams. Also, to emphasize the deleterious nature of an incorrect prediction in either direction, an incorrect prediction was taken as nullifying a correct one.

To determine whether any particular amount of an element in a bryophyte indicated mineralisation, the analysis figures were arranged in order. Since in this particular survey, we knew that eight streams contained mineralisation, the top eight figures were taken as being a positive prediction. The actual scale used, was as follows; if mineralisation or non-mineralisation was successfully indicated by the elemental content, a grade of +2 was awarded, and if the prediction was incorrect, the grade was -2. In the two cases where mineralisation was doubtful, the grades were +1 and -1 respectively. The allotted grades, out of a maximum total of +25, and minimum total of -21 are shown in table IV.3. Also included in the table are predictions from data by Wodzicki (1959b) on the stream water content.

The reliability indices allotted from Games Theory show that the apparent reliability of the various techniques used for uranium prospecting decreased in the sequence; uranium content of peat adsorbers, and; uranium, beryllium, lead and copper concentrations in bryophytic material. Of these values, only the uranium content of peat samples, and of bryophytic material are of worthwhile significance, and indirect indication of uranium mineralisation by pathfinders such as beryllium, copper

Table IV.3.

Elemental concentrations in bryophytes and stream waters from the Lower Buller Gorge region of New Zealand

Stream serial No. and name	Uranium content of peat "adsorbers" (ppm in ash)	Uranium content of water (ppb)*	Mean elemental content of bryophytes (ppm in ash)				Known mineralisation in catchment area
			Bc	Cu	Pb	U	
1	10.5	-	82	37	910	14.3	None
2	18.6	3.25	69	45	296	19.5	None
3 (Tiroroa Ck)	18.6	2.30	41	14	120	16.8	Weak
4	9.3	-	33	20	230	8.8	None
5	9.4	-	36	17	345	12.1	None
6 (Batty Ck)	9.0	1.4	30	6	140	4.7	None
7 (Big Tick Ck)	57.0	-	109	78	510	11.2	Weak
8 (Jones Ck)	324.0**	-	33	143	260	52.0	Strong
9 (Hornfels Ck)	15.6	-	92	42	410	12.8	Strong
10 (Centre Ck)	18.3	-	93	30	293	18.7	Strong
11 (Robyn Ck)	33.0	-	53	14	690	68.0	Strong
12	36.0	-	65	60	650	86.0	Strong
13	75.0	0.80	43	140	180	6.0	Weak
14	4.0	0.65	13	61	150	0.7	None
Reliability index (maximum +25)	+17	-2	+8	+3	+5	+13	-

* Data from Wodzicki (1959a).

** High value, due to subsequent contamination of catchment area by mining operations.

and lead was therefore not successful. It is of interest that the stream water analyses were not a very reliable guide to the presence of mineralisation compared with the peat adsorbers, or the bryophytes themselves.

D. Discussion.

Evaluation of the technique is still not easy, but we may say that it appears to be more successful than stream-water sampling under these particular conditions.

Advantages of this method are that it can potentially detect buried deposits and that the concentration factors are enormous compared with normal biogeochemical accumulations. A number of leaves taken from shrubs growing near the streams in which the mosses had uranium contents as low as 0.07 ppm compared with mean contents of 20 ppm for the mosses. This makes analysis relatively easy. Even more marked are the concentration factors, when compared with those for higher plants. Plant/soil ratios for uranium are usually about 0.2 (see section V), but the concentration ratios (moss/stream water), are about 10,000.

There are a number of disadvantages. First there is inherent variability in the bryophyte samples themselves, even from a single stream (which may be due to difficulty in separating different species). This variation can be reduced by taking the mean of a number of bryophytes from each stream, as was done in this survey, but streams containing large numbers of bryophytes are usually in shady areas with many steep and slimy waterfalls, rendering collection hazardous and slow. This disadvantage of the bryophytes does not apply to the peat adsorbers, but when the latter are used, the time needed to give a measurable amount of uranium is short, and it might be expected that variability in flow rates would produce less accurate prediction. That this is not shown by the figures in table IV.3., suggests this may not be an important disadvantage.

The second disadvantage, which applies specifically to the peat adsorbers, is the length of time (seven days) they must be left in contact with the water to achieve equilibrium. This may not be very practical in an extended prospecting programme, but if completely-buried ore bodies are being sought, the length of time might not matter, since there might be very few other means of detecting such mineralisation.

The third disadvantage is the possibility of there being no mineralisation within a catchment area, but simply disseminated uranium, of a grade far from economic, but producing very high levels of uranium in the water of the stream. This disadvantage is shared with stream-water sampling itself, and is usually not serious, since it will be so clearly correlated with the rock-type.

The fourth disadvantage is the difficulty of fixing a level at which the amount of uranium in a bryophyte or peat adsorber becomes significant. This problem is faced in every biogeochemical and geochemical prospecting survey, and can often be solved by a preliminary survey of known areas, and general knowledge gained over a number of surveys. Beyond this point, statistical methods should be resorted to.

One project, unfortunately beyond the scope of this thesis, is potentially capable of yielding more specific information. The bryophytic material is fairly specific as a chelator of uranium, but is is possible to obtain chelators which are still more specific. Some compounds in this category are insoluble resins, and include a polymer containing gallic acid and phenol (Hojo et. al., 1958), one containing oxidised diethylchlorophosphine and chlorinated polyethylene (Fielding, 1961), and various polyarsonic acids

containing resorcinol (Sani, 1966), and 4- hydroxystyrene (UKAEA, 1960). If resins like these were used in a similar manner to the peat, high specificity, and improved data might result.

The ideas expressed in this section of the thesis, need not be limited to uranium. Many other cations are adsorbed by moss material and peat, and selective chelators could certainly be synthesised for any given cation. These techniques are certainly in their infancy, but there does not seem a major reason why they should not, in time, become further useful tools for the prospector.

SECTION V

STUDY OF

RADIOACTIVE ISOTOPES

BY GAMMA-RAY

SPECTROMETRY

A. Introduction.

During the past decade, the subject of radioclements in plant and soil material has received increasing attention from many workers in different disciplines. This interest has arisen partly from the use of plants as a guide to mineralisation, partly from studies in natural environmental radiation, and partly from the monitoring of fall-out products.

Plants absorb different elements to differing degrees, and table V.1. adapted from Menzel (1965), gives concentration factors for a range of elements.

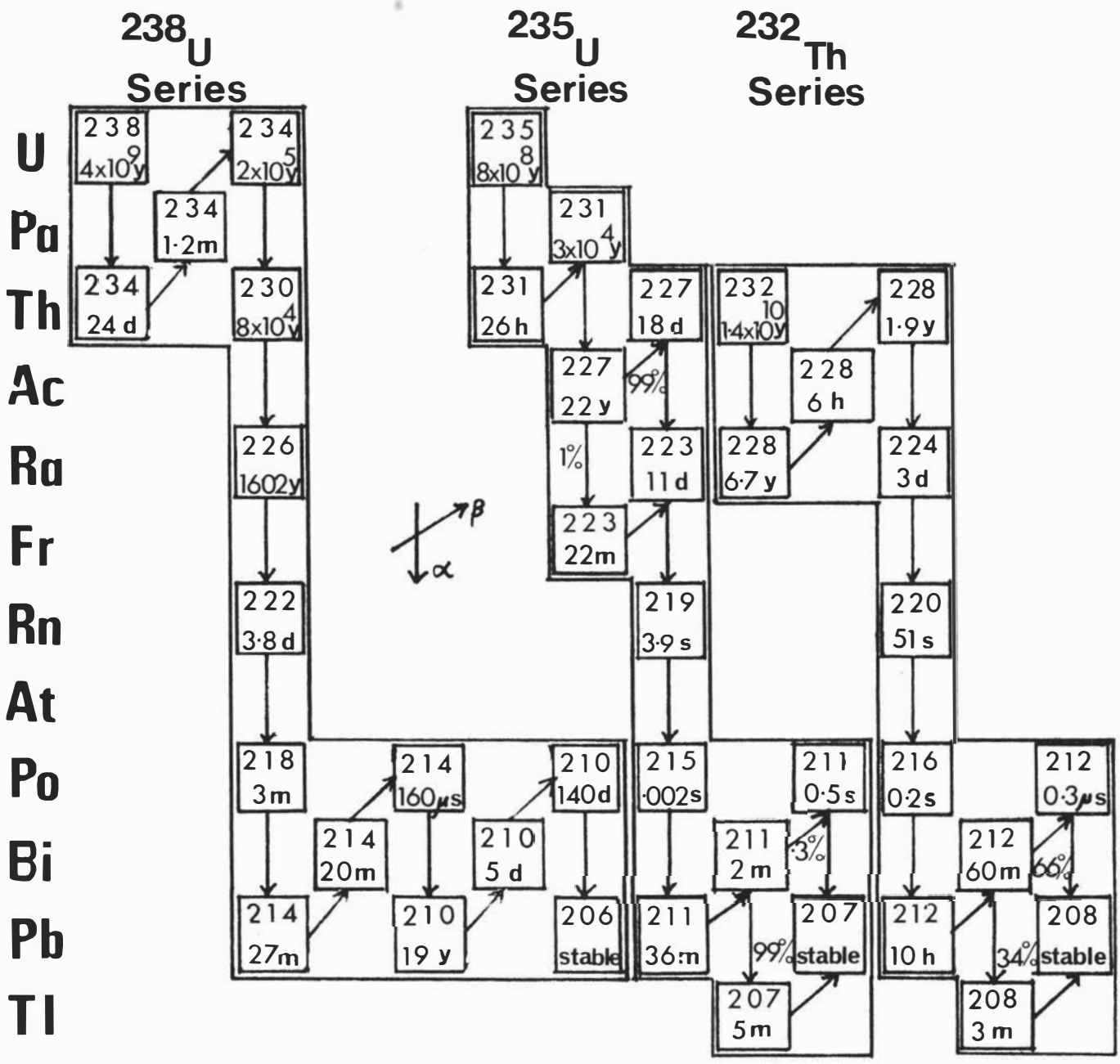
Cannon (1960a) and subsequently various workers in the Soviet Union (e.g. Botova et al. 1963) showed that many plants absorbed uranium, and used this as a basis for prospecting as described in Section III. Since this technique involves many routine uranium assays, attention was initially directed to the use of alpha counts as a measure of uranium, and as a type of measurement that could be relatively easily automated (Anderson and Kurtz, 1955, 1956). From fig.V.1. it may be seen that many radioelements emit alpha, particles, and if any of these are absorbed in large quantities by vegetation, the method may not give a true measure of the uranium present in the plant, and hence, indirectly, in the soil. An investigation of which radioelements are present in plant tissue seemed very important for an understanding of the information yielded by alpha-counting. Some information was already available from the literature, on the types of radioelements absorbed, but the data were usually qualitative.

The above literature has adequately established that uranium is absorbed by plants, but there is no similar information on the accumulation

Table V.1. Plant/soil ratios for various elements.

(Plant concentrations expressed on dry weight basis.)

Concentration factor	100-100	1-100	0.1-1.0	0.01-1.0	less than 0.01
Elements in that range	K,Rb,N,P, S,Cl,Br, Na,Li	Mg,Ca,Sr, B,Se,Te, Mn,Zn,Mo	Ba,Ra,Si F,I,Co,Ni, Cu,U	Cs,Be,Fe, Ru	Sc,Y,Zr,Ta, W,Ce,Pm,Pb, Pu,Sb,Bi,Th, Po


238 U Series
235 U Series
232 Th Series
U
Pa
Th
Ac
Ra
Fr
Rn
At
Po
Bi
Pb
Tl
238 4x10^9 y
234 2x10^5 y
234 1·2m
234 24 d
230 8x10^4 y
226 1602y
222 3·8 d
218 3m
214 160μs
214 20m
210 140d
210 5 d
214 27m
210 19 y
206 stable
235 8x10^8 y
231 3x10^4 y
231 26h
227 18 d
227 22 y
99%
1%
223 11 d
223 22m
219 3·9 s
215 ·002s
211 0·5 s
211 2 m
·3%
211 36m
99%
207 stable
207 5m
232 1·4x10^10 y
228 1·9 y
228 6 h
228 6·7 y
224 3 d
220 51 s
216 0·2 s
212 0·3μs
212 60m
66%
212 10 h
34%
208 stable
208 3 m
β
α

of protactinium.

Thorium is only slightly absorbed by higher plants. Baranov and Kunasheva (1954) claim to have demonstrated absorption of thorium isotopes, but their claim must be suspect, on the grounds of the very scanty evidence presented. Roser (1966) found no ^{232}Th in plants, and Vernadskii et al. (1937) found no ^{228}Th in duckweed, although water contained measurable quantities of this nuclide. In 1967, Gvozdanovic et al. found ^{228}Th in a specimen of oak which was four hundred years old, and inferred that some ^{232}Th must have been absorbed for significant quantities of the daughter to be still present. However, the major contribution to this field appears to be by Verkhovskaya et al. (1967), who clearly showed that although the concentration factor was very much less than one, the thorium isotopes that are absorbed are deposited in the oldest tissues, such as branches. These authors also found that mosses had much higher concentration factors.

Part of the difficulty of investigation of thorium accumulation lies in the strong absorption of radium, which often makes it hard to decide whether thorium present in living tissues has been absorbed, or has originated by decay.

The accumulation of actinium by plants has not previously been studied.

It is well known that radium occurs in Brazil nuts (Hill, 1962; Penna-Franca et al., 1968; Gabay and Sax, 1969), and it is even better known that almost all plants absorb this element, probably because of its chemical resemblance to calcium (Kunasheva, 1944; Penna-Franca, 1959; Kovalevskii, 1962; Tso et al., 1964; Kovalevskii, 1965; Makarov, 1965; Mistry et al., 1965; Penna-Franca et al., 1965; Raikov et al., 1966). This absorption is especially evident when the plants are young (Roser, 1966; Tso et al., 1968). Seasonal variation also occurs (Brunovskii and Kunasheva, 1935). The absorption is usually quantitatively greater than that of uranium.

Francium has too short a half-life to be absorbed per se, but decays via beta-emission to ^{223}Ra, which is absorbed quite readily.

Radon as a gas, presumably can diffuse into plants, but Tso et al.(1966) have shown that this process is negligible as a source of uptake of radioelements.

Polonium has been the subject of intense investigation in the past few years, in view of its role as a potential carcinogen. The element was detected by Radford and Hunt (1964) in tobacco, and since it volatilises at the temperature of a glowing cigarette, the danger was obvious. It had not been thought previously that polonium penetrated into the plant past the root system (Hill, 1960; Gulyakin and Korovkina, 1963). Tso et al.(1964), showed that the polonium in plants could not be accounted for by decay of absorbed ^{228}Ra, so Francis and Chesters (1967) suggested that it originated from absorbed ^{210}Pb. In the following year (Francis et al., 1968), postulated that the ^{210}Pb was fallout absorbed after foliar deposition. Tso et al. (1968), Tso and Fisenne (1968), and Watters and Johnson (1968),

however, showed that the ^{210}Pb present was not sufficient to account for the levels of ^{210}Po found, and concluded, for this particular plant, that polonium in the leaves is derived from that in the soil by direct absorption. This emphasises the danger of taking accumulation coefficients like those in table V.1 as absolute for all plant species, and the limitations of a literature survey such as this.

Bismuth has been observed in foliage, but the amounts are very low and are of the order of 1 ppm or less (Skulmowski and Wiercinski, 1966).

Lead, from the classic study of Hevesy (1923) and others (Keaton, 1937; Wilson and Cline, 1963; Tso et. al., 1966), is known to be absorbed by plants, though it seems most of it is fixed at root level. The amount reaching the leaves however, is sufficient to ensure the feasibility of biogeochemical prospecting (e.g. Nicolas and Brooks, 1969).

In general, it is known that lower plants like mosses and lichens are intense accumulators of most of the above elements (Grodzinskii, 1959).

It is theoretically possible to gain an idea of the isotopes present, and their relative amounts by alpha-particle spectrometry. However this involves the use of 'infinitely thin' sources, and in initial trials it was not possible to produce sources thin enough to give a spectrum containing sharp peaks. Extensive chemical purification would have been of great assistance, but this presupposes a knowledge of the very isotopes one wishes to detect. The same problems occur with beta-spectrometry, but the use of gamma-ray spectrometry avoids these difficulties.

The use of spectra produced by electronic transition in atoms, is a well established analytical tool. It is not so commonly realised that there is an analagous spectral technique possible, using gamma rays instead of visible light, and arising from transitions in the nucleus rather than electronic transitions. Just as the emission spectrum of an element is determined by the configuration of its electrons, so the spectrum of the gamma rays emitted is determined by, and is typical of, the configuration of the specific nucleus. A disadvantage of the technique is that only those elements that are radioactive may be easily used, and even some of those, like ^{238}U, emit such feeble gamma radiation that they cannot be detected. The chief advantage, is that no sample preparation is needed, owing to the ability of gamma rays to penetrate most materials without significant absorption.

In common with all spectral techniques, difficulty may be experienced if many emitting species are concurrently present, owing to the possibility of overlap of the different gamma spectra. This problem can sometimes be solved by improved resolution, and part of the work described in this section shows how this can make possible the detection of isotopes which previously could not be unequivocally identified.

For gamma spectrometry, the usual detector has been a large sodium iodide crystal doped with thallium. This system has a detection efficiency which remains high over a considerable energy range, but suffers from poor resolution, especially in the low energy region.

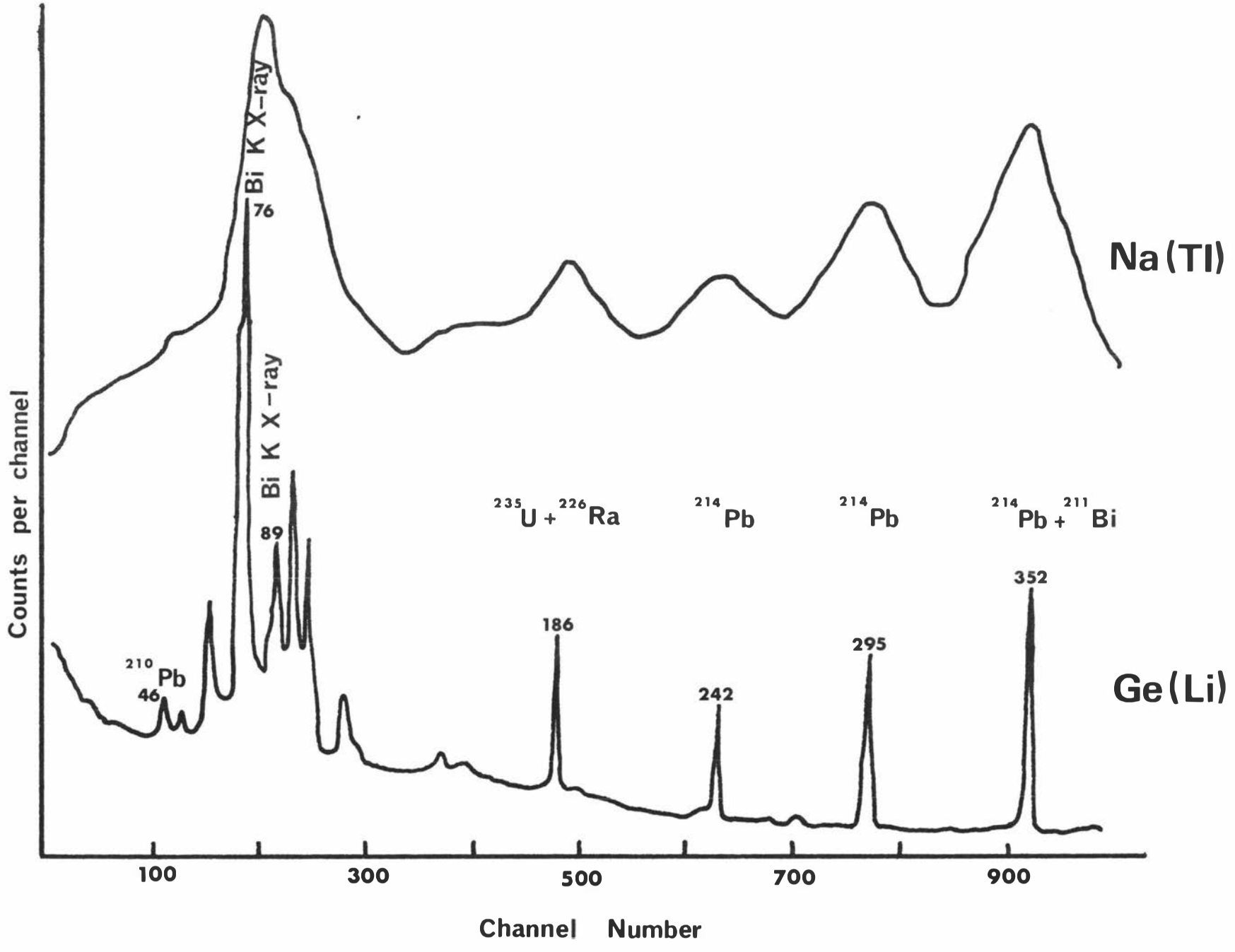
The use of lithium - drifted germanium crystals, has been described by Ewan and Tavendale (1964) and Coote (1967). Their resolution is usually about an order better than the sodium iodide type, and in the low

energy region extensive detail becomes visible (Potter et al., 1969). An example of this superior resolution is presented in fig. V.2., where the same sample of uraninite was counted using both systems. The detection efficiency of a germanium detector becomes rather low at high energies, but since counting above 360 keV was found to be superfluous, this disadvantage was not serious.

Most of the previously described work was not undertaken with germanium detectors, or indeed, by gamma-spectrometry at all. Radiochemical methods involving extensive chemical separations and subsequent alpha or beta counting were used. Gamma ray techniques would be much more elegant, if they could be shown to be applicable. The only such application to isotopes in the natural decay series appears to be by Parker (1962) who, in a notable paper gave the spectrum of ashed Darjeeling tea, with peaks he claimed were due to ^{210}Pb, ^{214}Pb, the Bi K x-ray, and ^{226}Ra. It will be shown later that such assignations without chemical evidence are doubtful, but in view of the fact that germanium detectors had not yet been developed, this was a very fine contribution.

Various studies have appeared on the gamma-ray emitters in fallout. In the majority of these, sodium iodide crystals were again used, and a large number of isotopes were detected, (Perkins, 1961; Burkholder, 1963; Phelps, 1967). Especially relevant to this part of the thesis is the almost universal detection of ^{144}Ce (Rickard et al., 1964; Anon, 1966), and ^{210}Pb (Beasley and Palmer, 1966; Kauranen and Miettinen, 1967).

In 1969, the author together with Drs. G.E. Coote and N.E. Cohen jointly developed techniques for the identification of gamma spectra peaks in the low-energy (< 200 KeV) range. Following the successful


Na(Tl)
Ge(Li)
Bi K X-ray
76
Bi K X-ray
89
^{210}Pb
46
^{235}U + ^{226}Ra
186
^{214}Pb
242
^{214}Pb
295
^{214}Pb + ^{211}Bi
352
Counts per channel
100
300
500
700
900
Channel Number

characterisation of the peaks, this method was used to study the nature of radioactivity in vegetation and this work is presented in this section together with the data for the characterisation of the gamma spectra.

B. Methods

1. Equipment

(a) dectector

A lithium-drifted germanium detector, volume 5 cm^3 was used. This is unusually small for this type of work, but proved completely adequate. The resolution was 2.1 keV (FHWM).

The detector was placed in an evacuated cryostat cooled by a copper rod dipping into liquid nitrogen, and positioned so that no absorbing material was between it and the front wall of the cryostat, which consisted of aluminium 0.025 cm thick.

(b) associated electronics

The output of the detector consists of current pulses whose intensity is dependent on the energy of the gamma ray striking the detector. These pulses are amplified, then sorted electronically by intensity into channels whose limits are determined by the operator, and whose number is determined by the design of the instrument.

In this work, the detector was coupled to a Simtec P-11 transistor preamplifier, and an Ortec 410 main amplifier. Pulse-height analysis was carried out by an RCL 256-channel analyser, but for a few very accurate calibrations a Kicksort 4096-channel analyser was used. Data output was in three forms. In the first, the number of pulses accumulating in the various channels were displayed simultaneously on an oscilloscope screen, making possible constant monitoring of the rate of accumulation of the spectrum. In the second, the number of counts in each channel could be automatically recorded via an associated electric typewriter, to be later plotted by hand, as a graph of the number of counts against the channel number. In the third form (applicable only to the Kicksort) output was via a punch onto paper tape.

The paper tape was used as input for automatic plotting of the data by a PDP-8 computer and associated XY-plotter. The systems used are depicted in figs. V.3 and V.4.

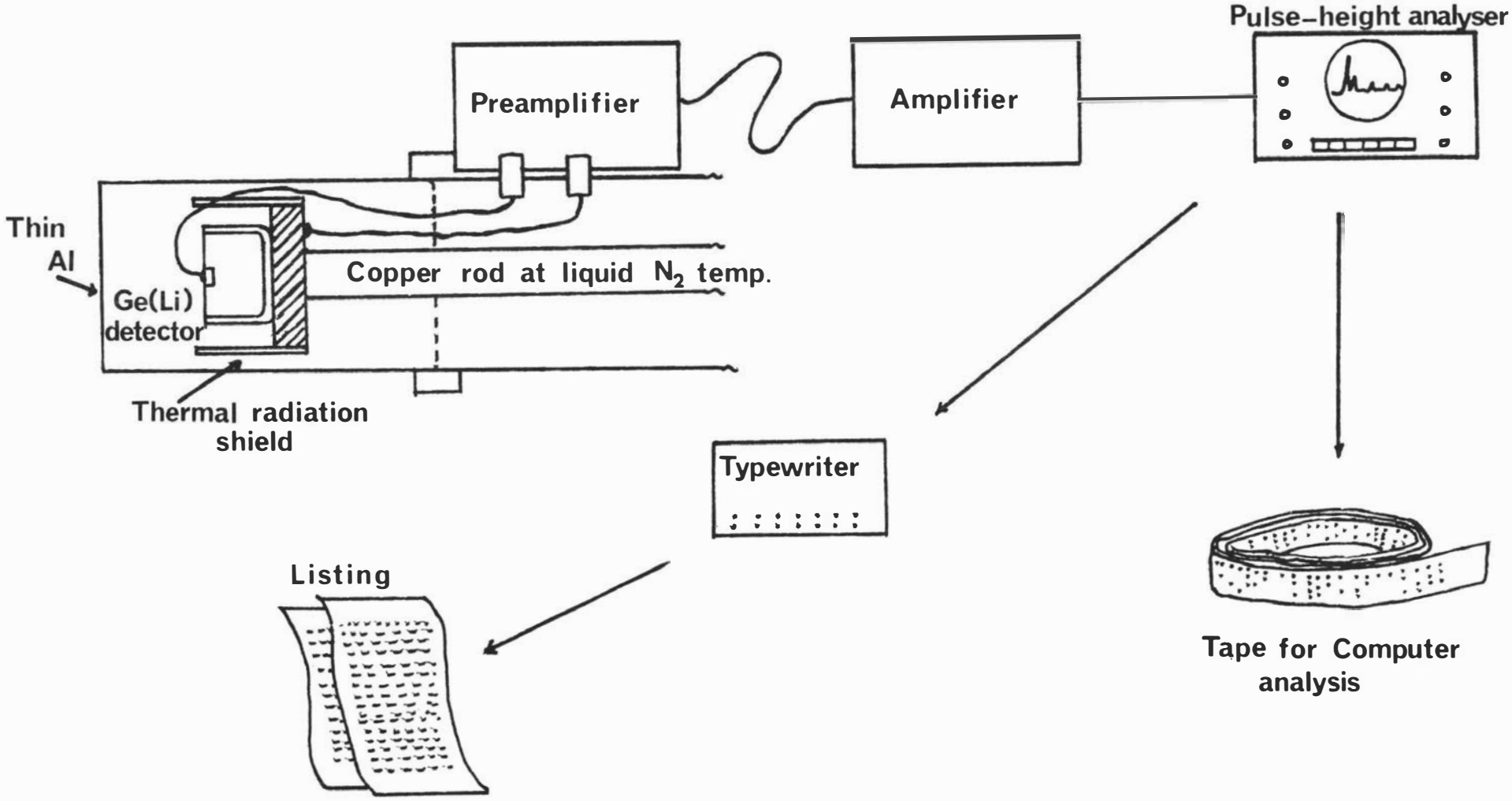
2. Counting procedure

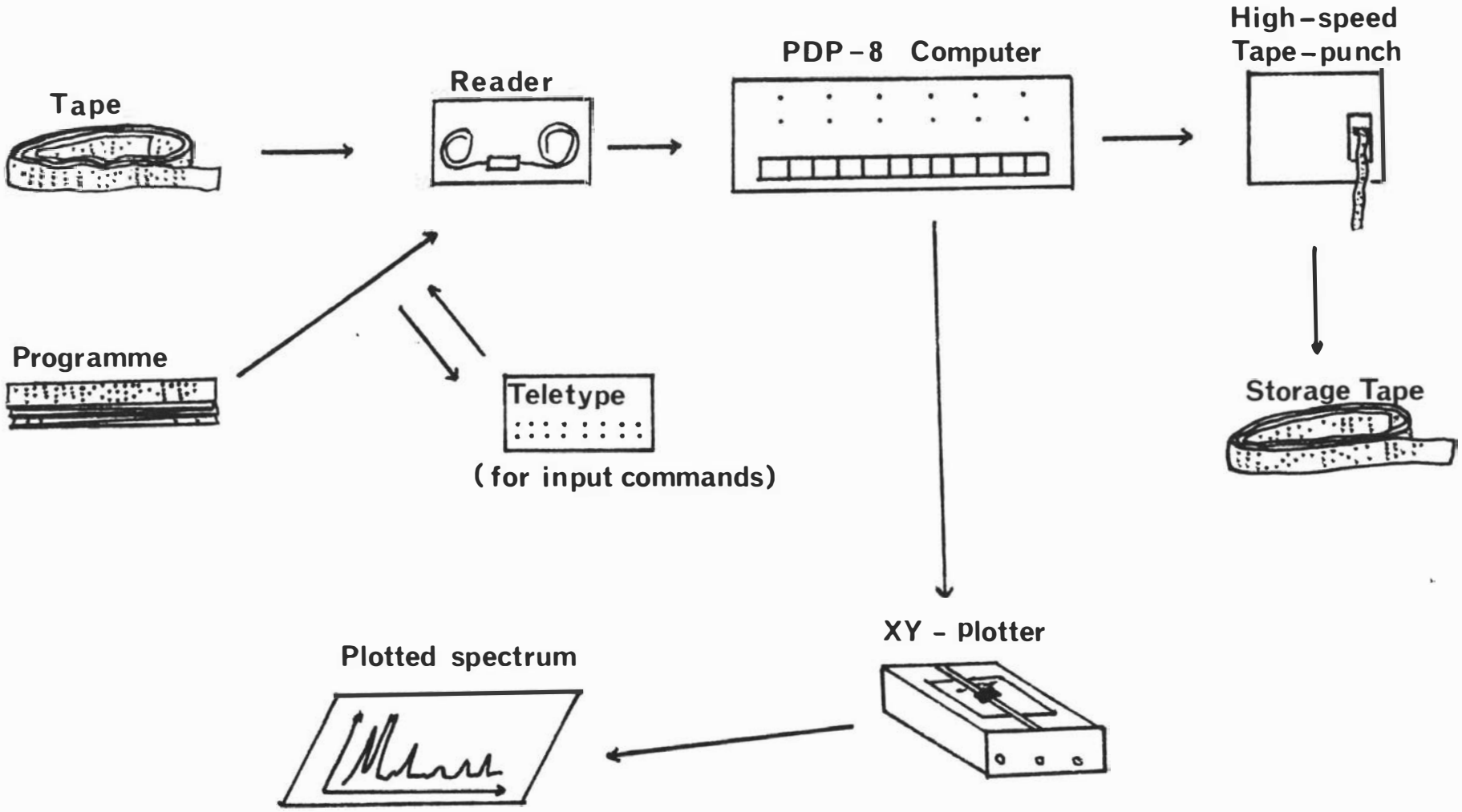
(a) sample placing

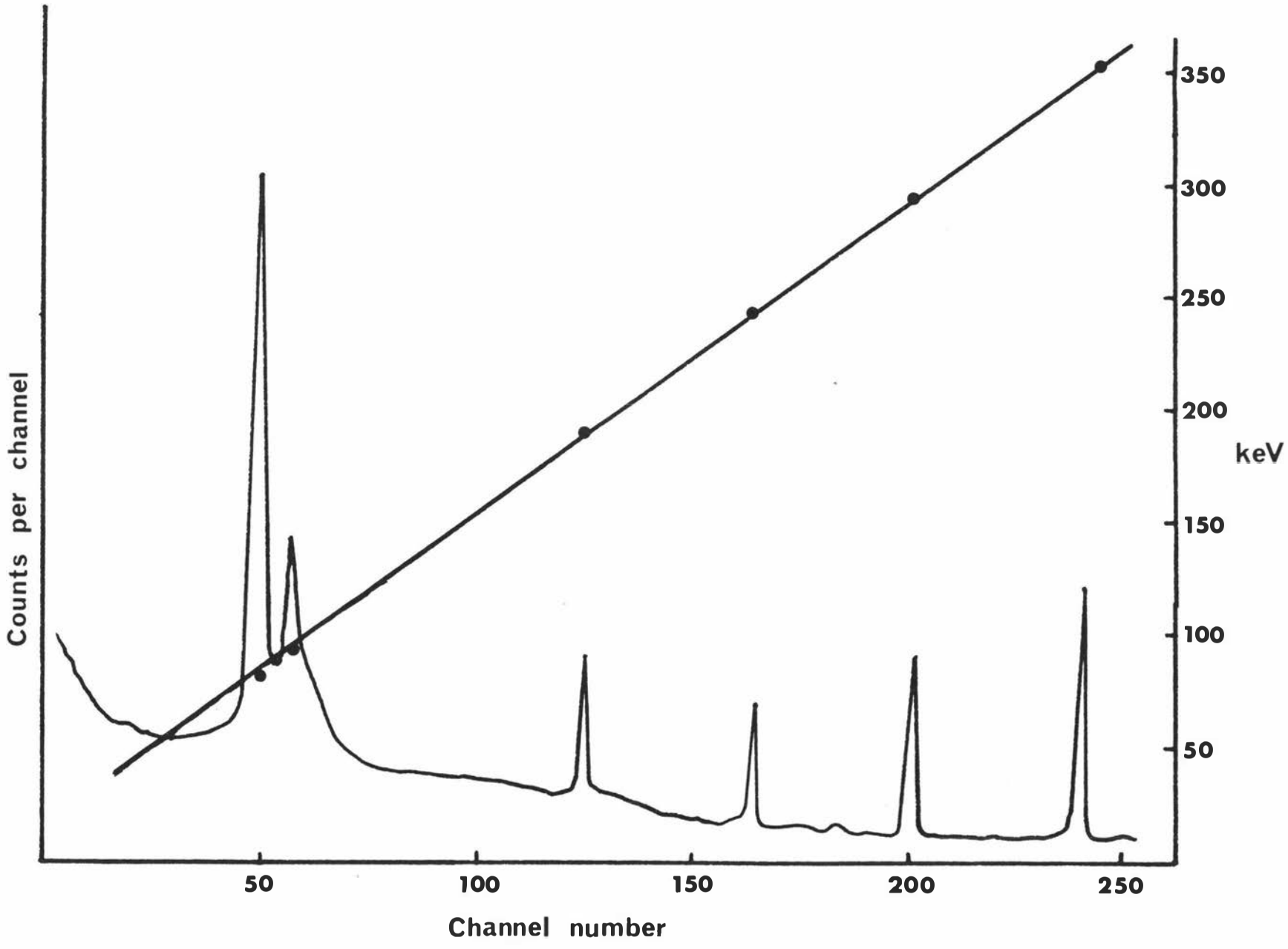
Five cm. of lead was used as shielding around the sample, until it was found that a spurious peak at 46 keV appeared in all spectra due to small amounts of ^{210}Pb in the lead. This was eliminated by shielding the detector from the lead with 1 cm. of perspex and thin copper and cadmium sheeting. The purpose of the cadmium was to reduce the background neutron flux from the electron accelerator targets (sixty feet away). Ash or soil samples of known weight were put in a polythene bottle, diameter 5 cm., and placed against the thin aluminium wall of the detector cryostat. As nearly as possible, the bulk and weight of all samples was made the same, when quantitative measures were taken, but since counting in containers of different materials and sizes made little apparent difference, this precaution to avoid bulk effects was possibly unnecessary. Counting times were sometimes as long as 3000 minutes, but most were about 1400 minutes.

(b) Calibration of the detector

It is necessary to know which energy range (in keV) a given channel is associated with. This was determined by accumulating the spectrum of a 5 μCi ^{226}Ra source from Amersham, the energies of its peaks being well known. Sufficient peaks are produced to give a good calibration curve from about 70- 352 keV, and the data are usually fitted very closely by a straight line. A typical calibration curve is given in fig. V.5. The electronics of the system were remarkably stable, and recalibration was


Pulse-height analyser
Preamplifier
Amplifier
Thin
Al
Ge(Li)
detector
Copper rod at liquid N_2 temp.
Thermal radiation
shield
Typewriter
Listing
Tape for Computer
analysis


Tape
Reader
PDP-8 Computer
High-speed
Tape-punch
Programme
Teletype
(for input commands)
Storage Tape
XY - plotter
Plotted spectrum

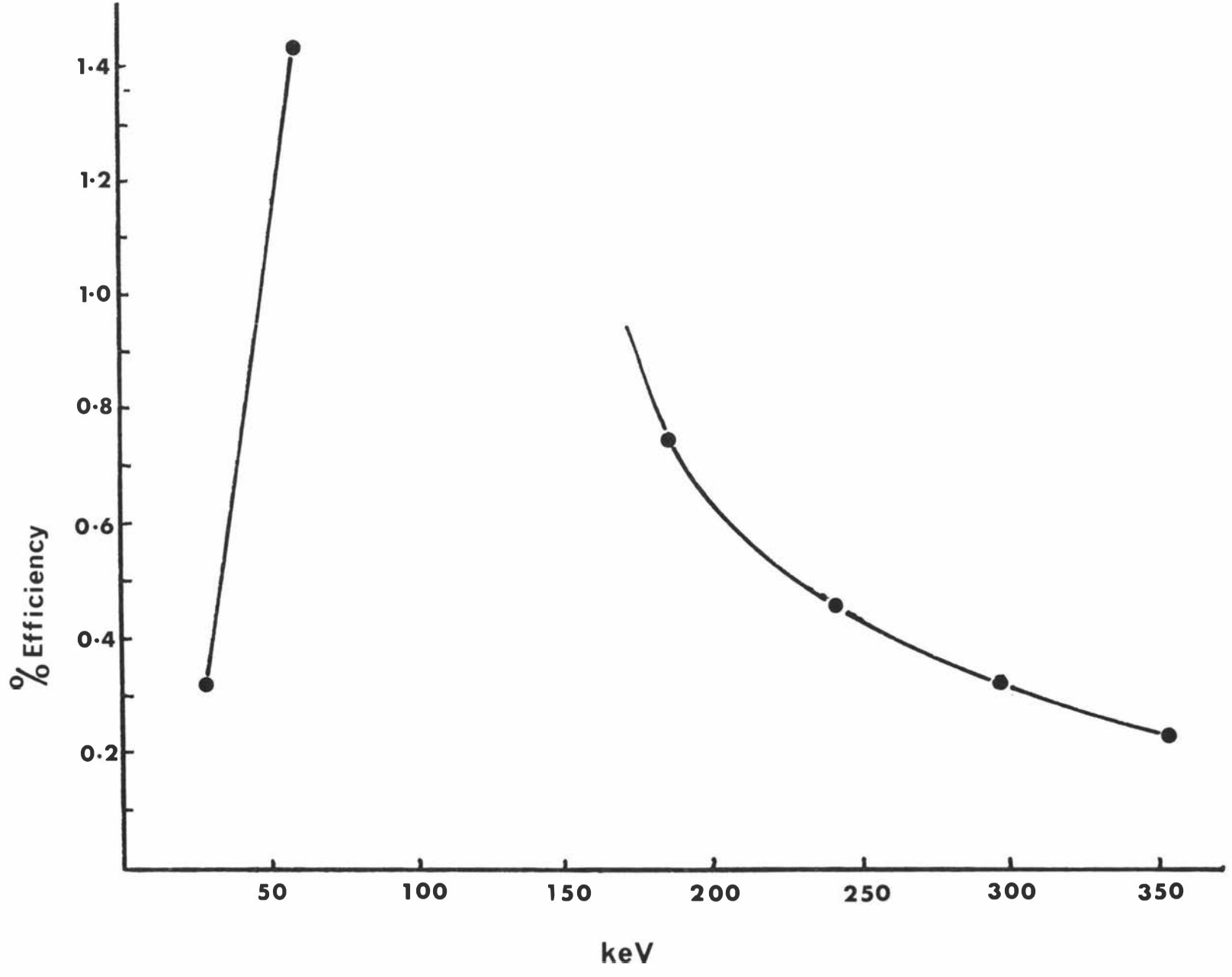

Counts per channel
350
300
250
200
keV
150
100
50
50
100
150
200
250
Channel number

rarely necessary more than twice a week.

It was mentioned earlier that germanium detectors are not so efficient in the high energy region. In practice, there is a consistent change in efficiency with energy, and to obtain figures for absolute intensity of gamma-ray emission, it is essential to find the efficiencies at a number of different energies. This is done by finding the relative efficiency at a number of points on the energy spectrum and then finding the absolute efficiency at one point, following which the absolute efficiency at all points may be found. The ratios of the intensities of the peaks in the ^{226}Ra spectrum are accurately known (Wallace, 1969), and these were used to construct a relative efficiency curve. By use of an IAEA ^{203}Hg standard of accurately known gamma-ray intensity, the absolute efficiency at 188 keV was found to be $(0.340 \pm 0.001)\%$, and the absolute efficiency curve obtained is depicted in fig. V.6. A ^{241}Am gamma-ray standard which contains two peaks of relatively low energy, was used in a similar manner to determine the absolute efficiency of the detector in the region 26 - 60 keV.

3. Identification of spectral peaks

Owing to the complexity of most spectra, it is seldom possible to assign isotopes to spectral peaks solely on the basis of energy of emission. Overlap of peaks is frequent, and chemical separations, or observations of time needed for decay, must be used as supplementary methods. This has been done too rarely, and one example of the difficulties that may arise is instanced in the work of Mathevon et. al. (1967) who identified (without chemical separations) the 186 keV peak as a combination of emission from ^{226}Ra and ^{230}Th. Chemical separations described later (subsections 3.(b) and 3.(c)), showed that the actual


1·4
1·2
1·0
0·8
0·6
0·4
0·2
% Efficiency
50
100
150
200
250
300
350
keV

isotopes responsible for the emission are ^{226}Ra and ^{235}U. This work appears to be one of the first attempts at rigorous assignation of isotopes to spectral peaks, and more are needed. Robert (1968) published data which became available in this country only after the completion of the work described in 3.(b) and 3.(c). His assignations of the peaks are mainly correct, but seem to have been arrived at more by intuition than systematic treatment.

(a) Energy considerations

A very few peaks can be identified by energy of emission alone. For this to be done successfully, there must be no other isotopes which emit in the same region. The only isotopes which could be identified this way are given in Table V.2.

Table V.2. Isotopes identified by energy of gamma-ray emission.

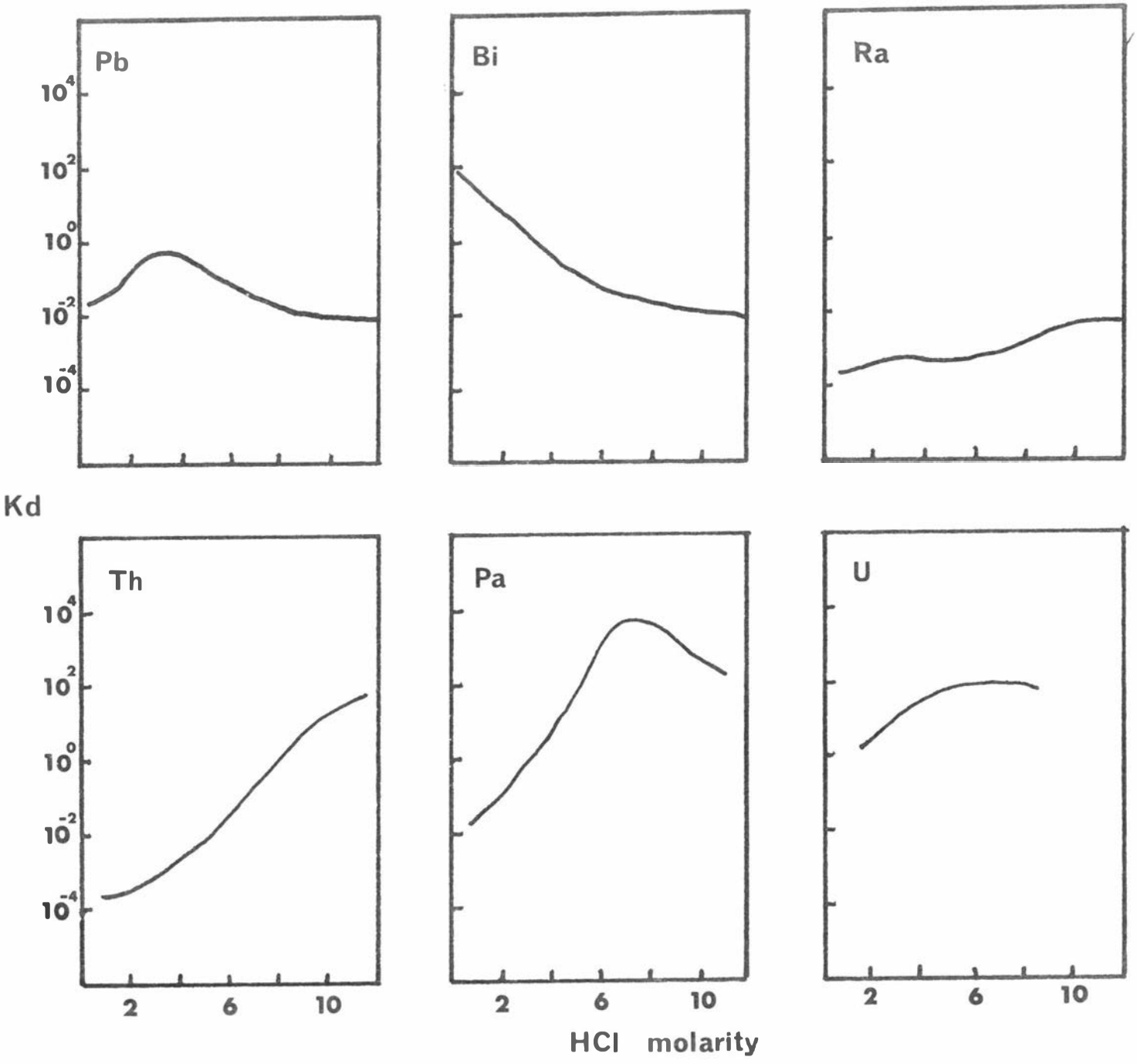
Isotope	Energy of peak (keV)
^{210}Pb	46
Th K x-ray.	106
^{235}U	162

Other peaks, following a survey of the literature (Lederer et al., 1969), were expected to have possible contributions from a number of isotopes.

(b) solvent extraction

The solvent extraction system, tri-n-butyl phosphate / HCl has been widely studied, and its extraction properties are well known for almost all elements and for various acidities (Sato, 1966; Ishimori et al., 1960). In fig. V.7. are shown the extraction properties of most of the radioelements in the decay scheme of fig. V.1, and the potential separation that could be achieved.

A specimen of uraninite dissolved in 2.5M HCl was extracted with tri-n-butyl phosphate, and the non-aqueous layer was then counted, and recounted after an interval of 18 hours giving spectra as in fig. V.8. The changes in height of some of the minor peaks enables the assignations in Table V.3. to be made.


Pb
Bi
Ra
Th
Pa
U
Kd
10^4
10^2
10^0
10^{-2}
10^{-4}
2
6
10
HCl molarity

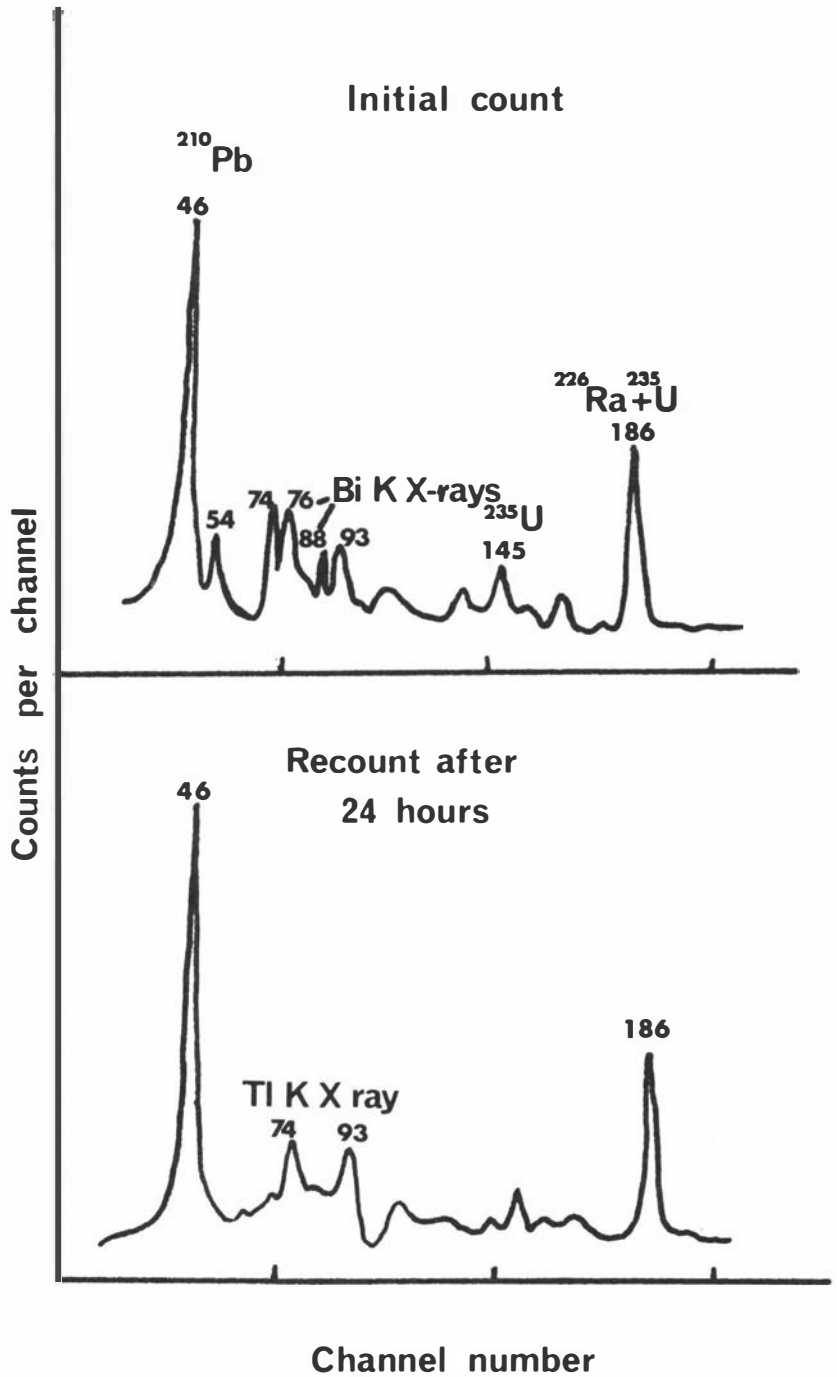

Initial count
210Pb
46
54
74
76
Bi K X-rays
88
93
235U
145
226Ra+235U
186
Recount after
24 hours
46
Tl K X ray
74
93
186
Counts per channel
Channel number

Table V.3. Isotopes assigned by solvent extraction properties.

Isotope	Energy of Peak (keV)	Reason for assignation
^{214}Pb	54	Found in non-aqueous layer, and decays rapidly because ^{226}Ra not extracted.
^{231}Th	74	Not initially in non-aqueous layer, increases due to ^{235}U presence. Increases too rapidly to be ^{230}Th.
Bi x-ray	77	Extracts into non-aqueous layer and vanishes overnight. Parent therefore not extracted.
"	88	As above.

(c) ion exchange

The spectrum of ^{235}U is complex (Mann et. al., 1966), and therefore the ion-exchange system of Korkisch and Arrhenius (1964) was used to separate uranium from other elements. Dowex 1 x 8 (100 mesh), chloride form, was packed in a column 15 cm. x 1 cm. giving a flow rate of 0.5 - 0.7 ml / min. It was converted to the nitrate form by passing 5 M nitric acid through the resin until the eluate no longer gave a positive test to silver nitrate. A solution of uranyl nitrate in 5 M nitric acid was passed through the column followed by 50 ml of the same solvent to remove any weakly-adsorbed elements. Thorium and uranium remained adsorbed. Thorium and other elements were eluted, by 35 ml of 6 M HCl, and uranium was eluted with 25 ml of 0.1 M hydrochloric acid, following which both fractions were counted, giving spectra as displayed in V.9. These spectra made possible assignation of the following peaks in Table V.4.

Table V.4. Isotopes assigned via ion exchange chromatography.

Isotope	Energy (keV)	Evidence
^{235}U	48	In uranium fraction.
^{234}Th	63	In non-uranium fraction. Decays over a few months.
^{231}Th	68	In uranium fraction and amount increases rapidly from ^{235}U decay.
^{231}Th	84	As above.
^{234}Th	93	In non-uranium fraction. Decays in a few months.
^{235}U	98	In uranium fraction.
^{235}U	110	In uranium fraction.
^{235}U	112 (broadens 110)	In uranium fraction.
^{235}U	144	In uranium fraction.
^{235}U	162	In uranium fraction.
^{235}U&^{226}Ra	186	In uranium fraction; also extraction into tri-n-butyl phosphate from 10 M HCl (which removes uranium) does not remove all of peak, therefore another component was present.

Further evidence for the compound nature of the 186 keV peak may be found in fig. V.10., (a and b). These show a ^{226}Ra source, and a uraninite source which will also contain ^{226}Ra. The 186 keV peak is higher in the uraninite spectrum relative to the other peaks which are due mainly to ^{214}Pb.

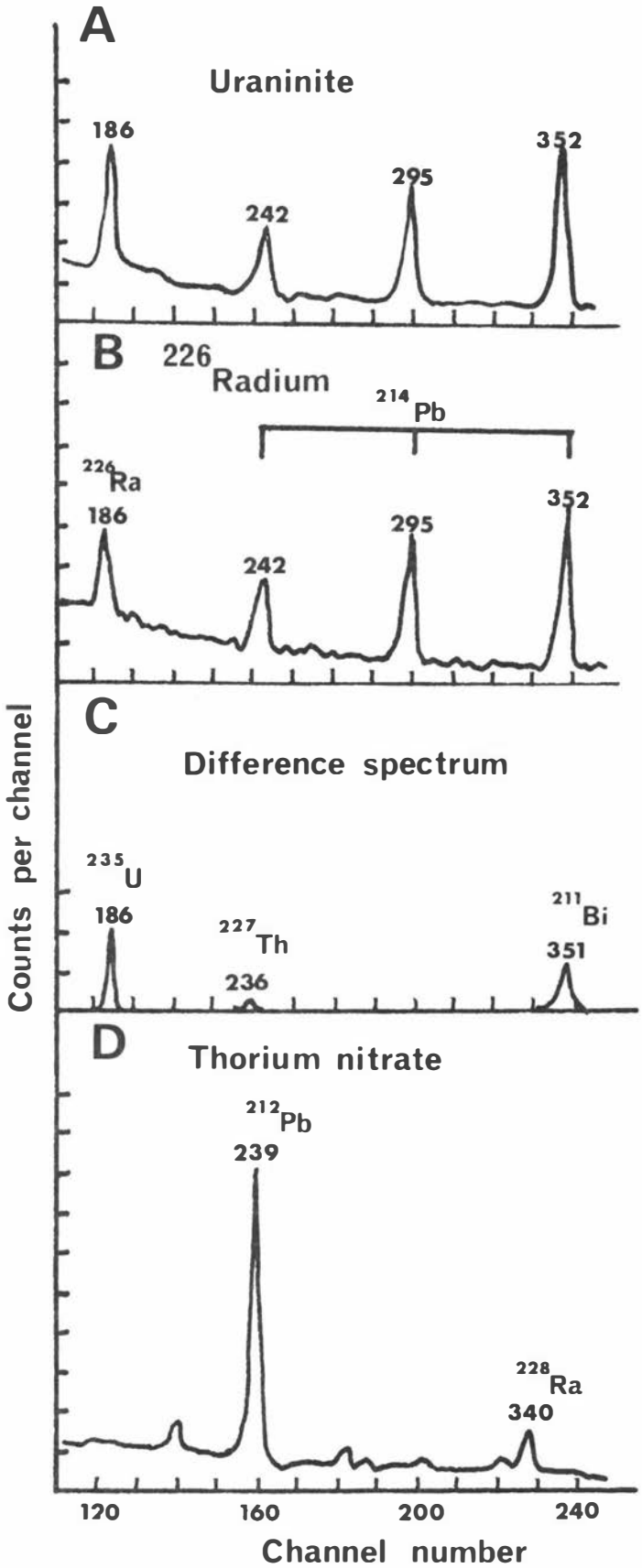
(d) a comparison of the spectra of uraninite and ^{226}Ra

This is depicted in fig. V.10 (c). A spectrum of uraninite was accumulated for three hours, then the uraninite specimen was replaced with a ^{226}Ra source, and via special circuitry in the RCL-256 multi-channel analyser, the accumulating ^{226}Ra spectrum was carefully subtracted from the uraninite spectrum until most channels just contained zero counts. The resultant spectrum proved that the 186, 242, and 352 peaks were compound, the other contributions being due to ^{235}U, ^{227}Th, and ^{211}Bi respectively. The 295 keV peak did not appear to contain significant interference from any other isotopes. A further possible contribution to the 242 peak is shown in the spectrum of thorium nitrate (fig. V.10.(d).); in practice this contribution was found to be the major interference, and the interference from ^{227}Th was minimal - especially in plant material.

(e) application of low-energy gamma-spectrometry to biogeochemistry.

Identification of a large number of isotopes is possible by gamma-ray spectrometry by use of the technique developed. The method was also successfully used to determine the percentage radioactive equilibrium in various radioactive ores (Coote, Cohen and Whitehead, 1970) but this will not be described in detail being outside the range of this thesis.

The isotopes of biogeochemical interest are ^{235}U, ^{228}Th, ^{223}Ra,


A
Uraninite
186
242
295
352
B
226 Radium
214 Pb
226 Ra
186
242
295
352
C
Difference spectrum
235 U
186
227 Th
236
211 Bi
351
D
Thorium nitrate
212 Pb
239
228 Ra
340
Counts per channel
120
160
200
240
Channel number

^{226}Ra, ^{228}Ra, and ^{210}Pb. These all have half-lives long enough to be absorbed by the plant, and can be detected either directly or by measuring other isotopes in equilibrium with them. Since 144 days elapsed between harvesting plant material and counting it, inspection of fig. V.1. shows that considerable equilibrium existed between many isotopes. Equilibrium was taken to mean a period sufficient to ensure that a parent isotope decays at the same rate as a daughter-isotope. This period is usually greater than six half-lives of the parent isotope. Thus it was possible to measure the amount of ^{226}Ra present, by measuring the 295 keV peak due to ^{214}Pb, which was in equilibrium with it. When the amount of ^{226}Ra was known, the amount of ^{235}U in the 186 keV peak could be found, by simple subtraction. Similarly, the known amount of ^{214}Pb enabled the amount of ^{212}Pb contributing to the 242 keV peak to be found. Since the ^{212}Pb was in equilibrium with ^{228}Th, the amount of the latter isotope could be found. A similar procedure with the 352 keV peak gave the amount of ^{227}Th present. In calculation of the absolute activities in pCi/g it is necessary to also take into account the efficiency of the different gamma-ray emissions, which differ with each gamma ray, even from the same isotope.

The calculations were complex, and a simple computer programme was written to avoid the tedium and inevitable mistakes due to fatigue, that a large volume of calculation brings. The programme is not given in this thesis, but the flow diagram for the calculations is given in fig.V.11.

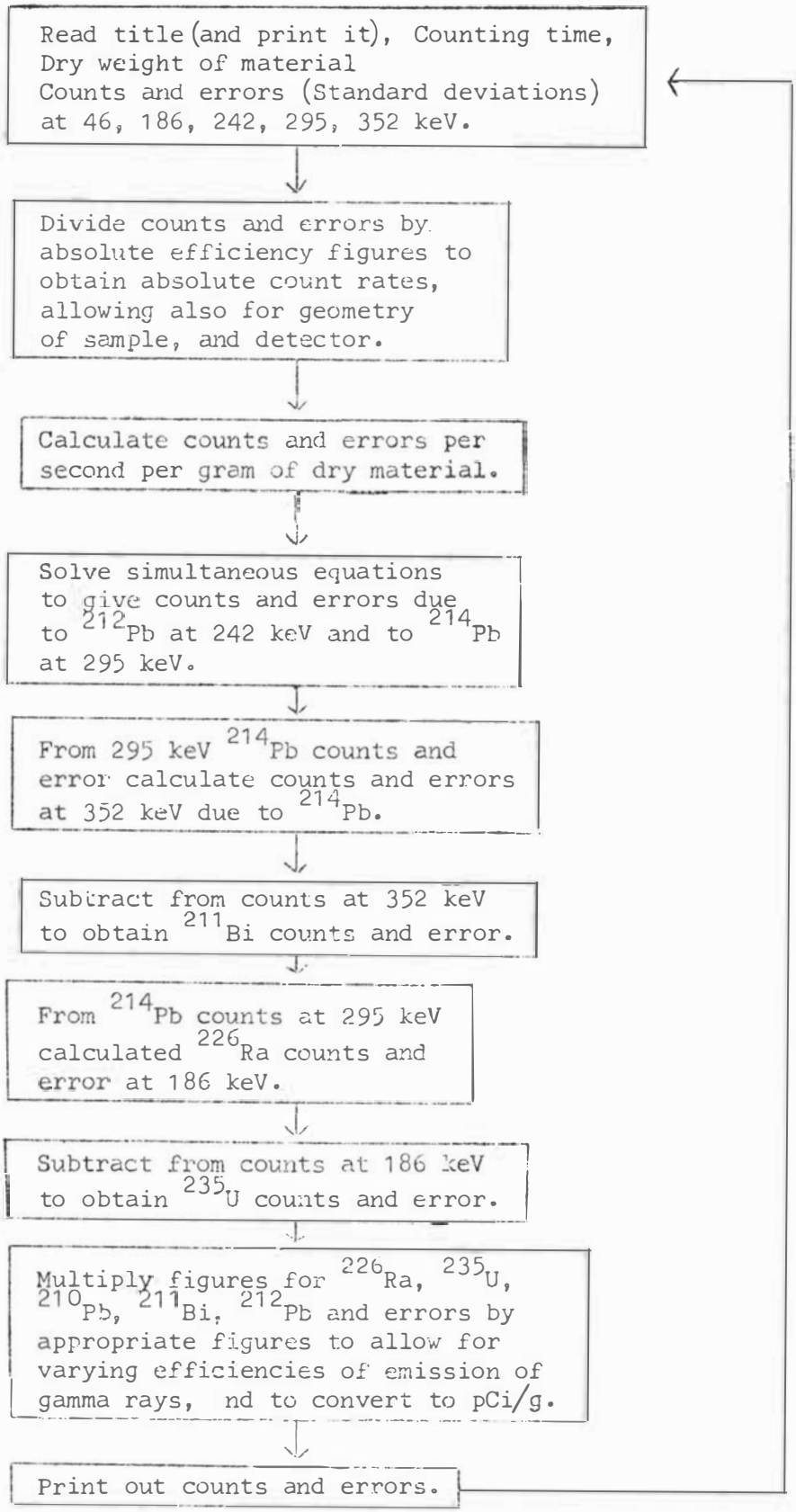


Figure V.11. Flow diagram for gamma-ray calculations.

C. Samples counted

Specimens of plants that leaves were collected from were; Weinmannia racemosa (kamahi), Nothofagus fusca (red beech), Coprosma australis, Quintinia acutifolia, Uncinia leptostachya, (sedge), Blechnum procerum (fern) and Marchantia berteroana (liverwort). Associated soils were collected in each instance. In the case of a ninety-year-old specimen of N.fusca, a cross section of the trunk was also taken, and the wood divided into five groups according to age. Specimens of Fissidens rigidulus, and Bryum blandum (aquatic bryophytes) were taken both from a mineralised stream and from an unmineralised stream. The lichen, Stereocaulon ramulosum found growing just underneath a uraninite vein was also included. All samples were counted as ash.

D. Results

1. Observed spectral types

Results are presented in table V.5. and show the activities found in the various specimens. These activities are expressed in terms of pCi/g dry weight, which gives a much better basis for comparison than an ash-weight basis, since these varied so widely in the specimens. The results are in terms of emission from the isotope actually measured, rather than any parents which may or may not be present. The error given in each case is the standard deviation as a percentage of the activity after correction for background and the previously mentioned allowance for the percentage of disintegrations known to produce the measured gamma ray, and the efficiency of the detector.

Fig. V.12. represents the different types of spectra recorded. Since the remaining spectra only differed quantitatively from these, it is superfluous to reproduce them.

The Uncinia is typical of most of the soils and vascular plants, with four prominent peaks corresponding to ^{226}Ra and ^{214}Pb (c.f. fig. V.10.). In this particular specimen, there was a substantial amount of uranium, and the 63 and 93 keV peaks due to ^{234}Th, a daughter of ^{238}U, can be clearly seen. The N.fusca leaf spectrum is included because of its two unusual peaks at 134 and 145 keV, which do not appear in the spectrum of its innermost trunk section, also shown in this figure. The trunk section has unusual peaks at 110, 122, and 277 keV, and is similar to some of the mosses. A spectrum of B. procerum is also included, because of its unusual peaks occurring (though in very much smaller amounts) only in one other sample; M. beteroana.

Table V.5. Radiometric data for soils and plants from the Buller Gorge uraniferous area

Species	Material	^{210}Pb			^{226}Ra			^{211}Bi			^{235}U			^{212}Pb		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Higher Plants																
B.procerum	Leaves	undetectable			589	6.0%	18.5%	450	20.3%	23.6%	238	28.5%	2.5%	37.2	11.4%	1.3
	Soil	1713	1.4%		1384	0.86%	17.8%	130	24.1%	2.8%	814	2.2%	3.5%	3.34	58.1%	0.(
C.australis	Leaves	74.5	4.4%		52.5	3.2%	24.6%	9.94	43.9%	7.8%	19.0	14.9%	2.9%	0.53	52.4%	0.2
	Soil	undetectable			29.2	32.1%	14.2%	undetectable			21.3	92.5%	3.5%	11.7	15.6%	7.(
Q.acutifolia	Leaves	1.68	109%		49.1	2.3%	48.4%	undetectable			6.84	30.2%	2.2%	0.56	34.7%	0.(
	Soil	19.0	49.1%		undetectable			18.4	64.0%	18.6%	17.1	22.9%	3.5%	3.31	14.9%	2.7
N.fusca	Leaves	3.67	49.2%		13.5	7.3%	2.1%	3.14	78.2%	8.3%	5.57	9.7%	3.0%	0.34	51.4%	0.(
	Soil	19.0	49.1%		undetectable			18.4	64.0%	2.2%	17.1	22.9%	4.2%	3.31	14.9%	3.1
U.leptostachya	Leaves	401	2.6%		258	2.0%	2.7%	undetectable			1228	7.14%	4.3%	0.11	787%	0%
	Soil	4961	1.5%		7586	0.53%	2.2%	undetectable			3707	1.2%	3.5%	21.5	29.9%	0.1
W.racemosa	Leaves	2.39	49.6%		4.68	13.9%	20.0%	1.4	128%	0%	2.46	54.4%	3.5%	0.06	217%	0%
	Soil	494	6.8%		370	4.6%	34.3%	154	28.2%	23.8%	388	8.46%	0.1%	316	0.91%	39.
Bryophytes etc.																
B.blandum (mineralised creek)	Whole plant	26.6	31.7%		12.1	37.9%	5.8%	28.2	40.7%	22.7%	19.3	48.6%	3.1%	2.33	37.5%	1.1
(unmineralised creek)	Whole plant	51.2	103%		55.3	53.5%	9.9%	60.6	111%	0%	63.7	97.9%	3.8%	18.6	30.7%	3.
F.rigidulus (mineralised creek)	Whole plant	133	33.8%		790	3.6%	56.8%	25.4	298%	0%	79.2	63.3%	1.9%	0.14	3340%	0%
(unmineralised creek)	Whole plant	6.79	86.2%		2.21	134%	0%	0.76	985%	0%	59.8	9.6%	4.3%	1.75	20.4%	0..
Lichen, Stereocaulon sp.	Whole plant	288	60.6%		358	28.8%	10.0%	81.8	141%	0%	417	50.3%	3.9%	51.2	38.1%	1..
M.berteroana	Leaves	1432	4.7%		1384	2.5%	12.5%	326	28.8%	4.9%	1210	5.4%	3.6%	11.7	50.6%	0.
	Soil	1261	1.7%		1291	0.88%	16.3%	undetectable			876	2.3%	3.7%	undetectable		

A : activity of sample in pCi/g (dry weight). B : coefficient of variation.
C : percentage contribution of isotope to total α activity of sample.
* Calculated from the ^{235}U data.

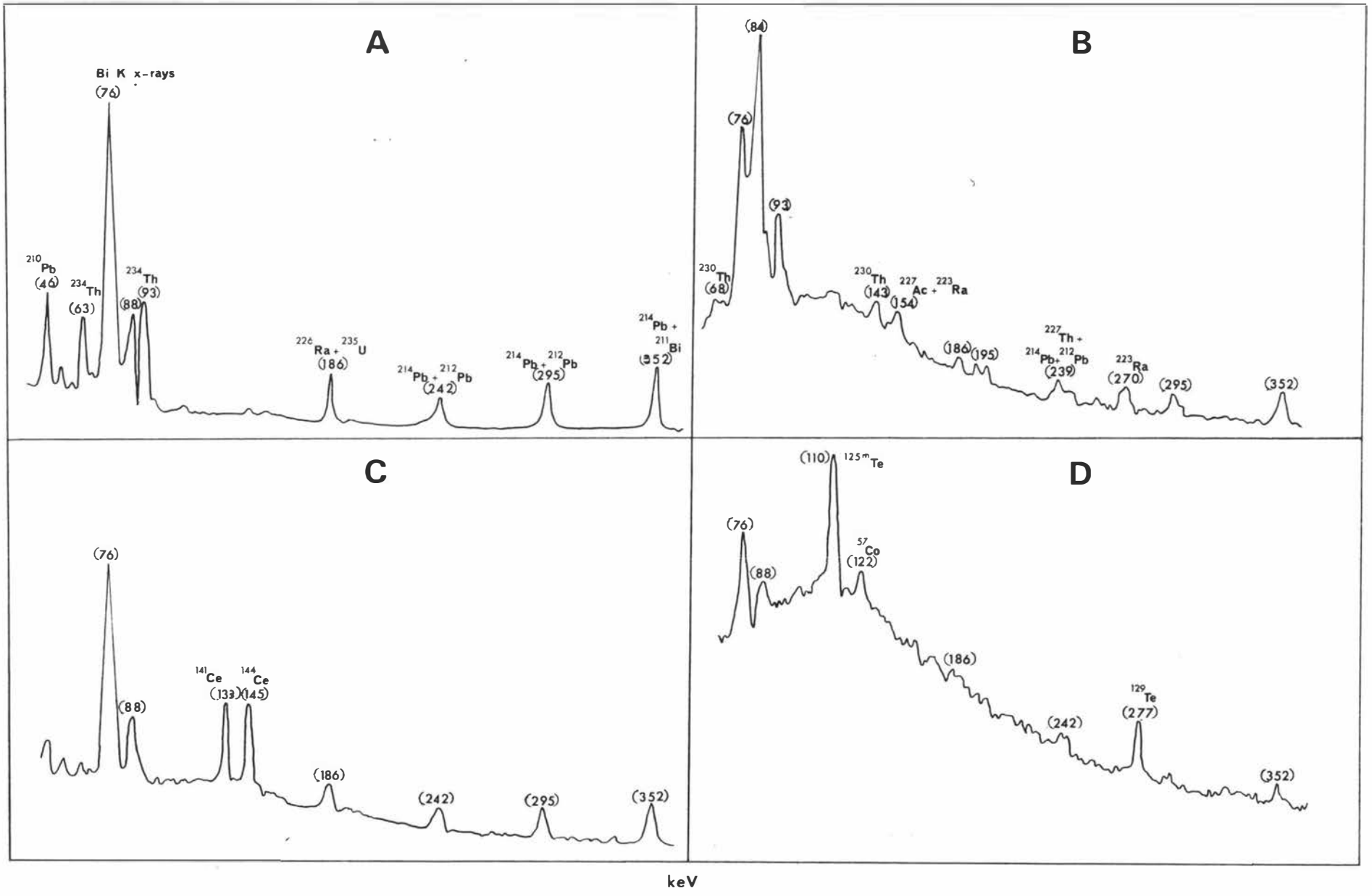

A
Bi K x-rays
(76)
210Pb
(46)
234Th
(63)
234Th
(88)
(93)
226Ra + 235U
(186)
214Pb + 212Pb
(242)
214Pb + 212Pb
(295)
214Pb + 211Bi
(352)
B
(84)
(76)
(93)
230Th
(68)
230Th
(143)
227Ac + 223Ra
(154)
(186)
(195)
227Th +
214Pb + 212Pb
(239)
223Ra
(270)
(295)
(352)
C
(76)
(88)
141Ce
(133)
144Ce
(145)
(186)
(242)
(295)
(352)
D
(110)
125mTe
(76)
(88)
57Co
(122)
(186)
129Te
(277)
(242)
(352)
COUNTS
keV

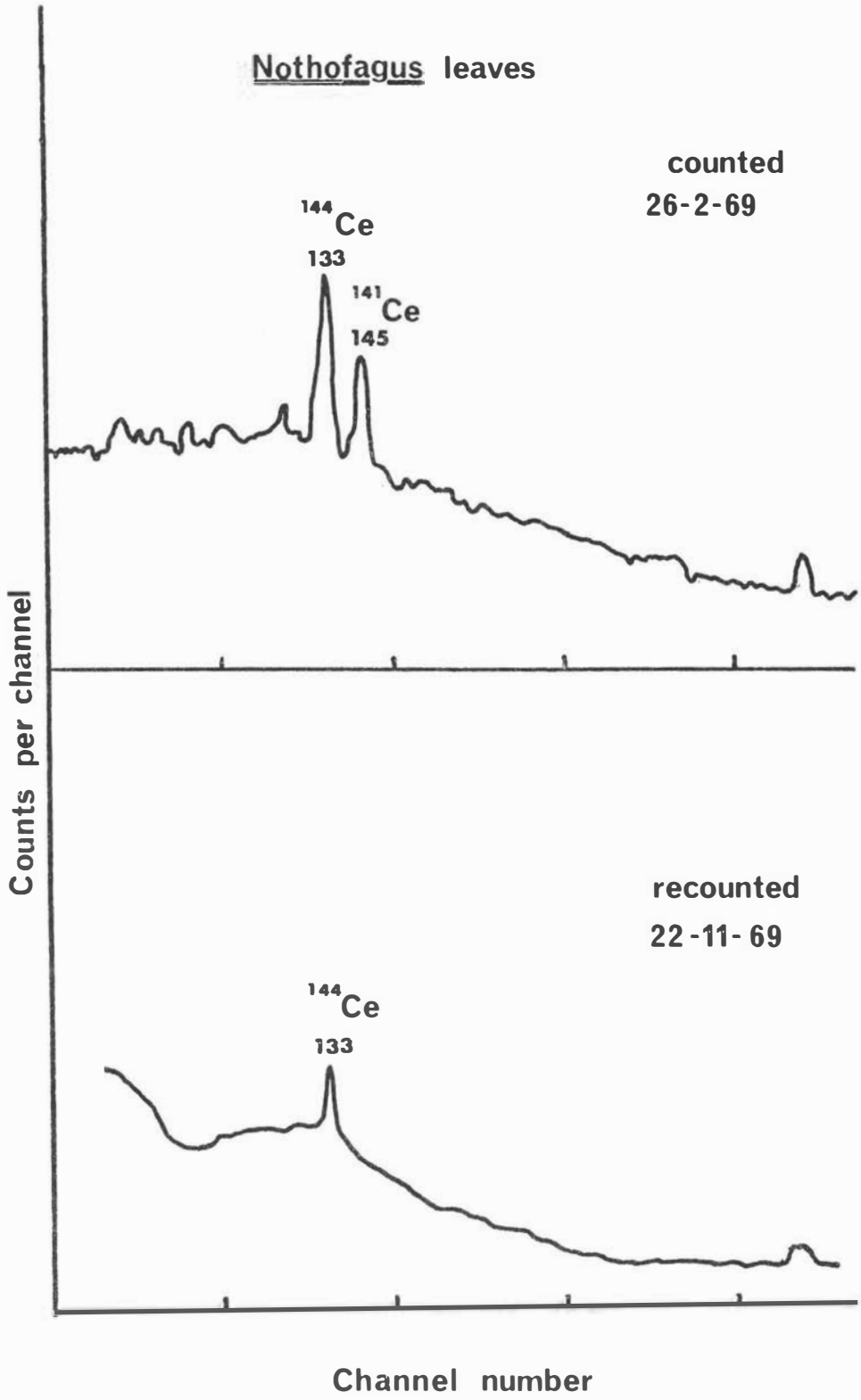
Figure 6

2. Fission products in plant material

The spectra for N.fusca, and innermost annual rings of N. fusca in fig. V.12., show a number of extraneous peaks which could not be accounted for by isotopes in the natural decay series. For example, the 134 keV peak is not known to be emitted from any naturally-occuring isotope, to any significant extent.

The presence of fall-out was suspected, and samples of beech leaves were therefore collected from the Matukituki Valley, (known to be unmineralised), about 400 miles south of the Buller Gorge, but receiving a very similar rainfall. Fig. V.13. gives the spectrum of these leaves, and the spectrum when recounted several months later. The peaks observed, and the decreases in height are consistent with the assignation of the two well known fission products ^{144}Ce and ^{141}Ce respectively. Other evidence in support of this assignation comes from the work of Gulyakin et. al. (1968) who showed that ^{144}Ce is not absorbed by plants from the soil, because of the extreme strength of its binding, and from the work of Gabay et. al. (1965), who showed that the predominant mechanism of uptake is foliar absorption. No ^{210}Pb is seen in leaves from the unmineralised area, which means that any of that isotope observed in the specimens from the mineralised zone must have originated either from decay of ^{226}Ra within the plant, or by direct absorption from the soil.

The unusual peaks in the innermost rings of N.fusca are a much more difficult problem, since the tree was ninety years old, and no fall-out was being produced at that time. One would not expect substantial quantities of fall-out products in such old tissues, unless they are very much more metabolically active than is generally supposed. There has appeared a study


Nothofagus leaves
counted
26-2-69
144Ce
133
141Ce
145
recounted
22-11-69
144Ce
133
Counts per channel
Channel number

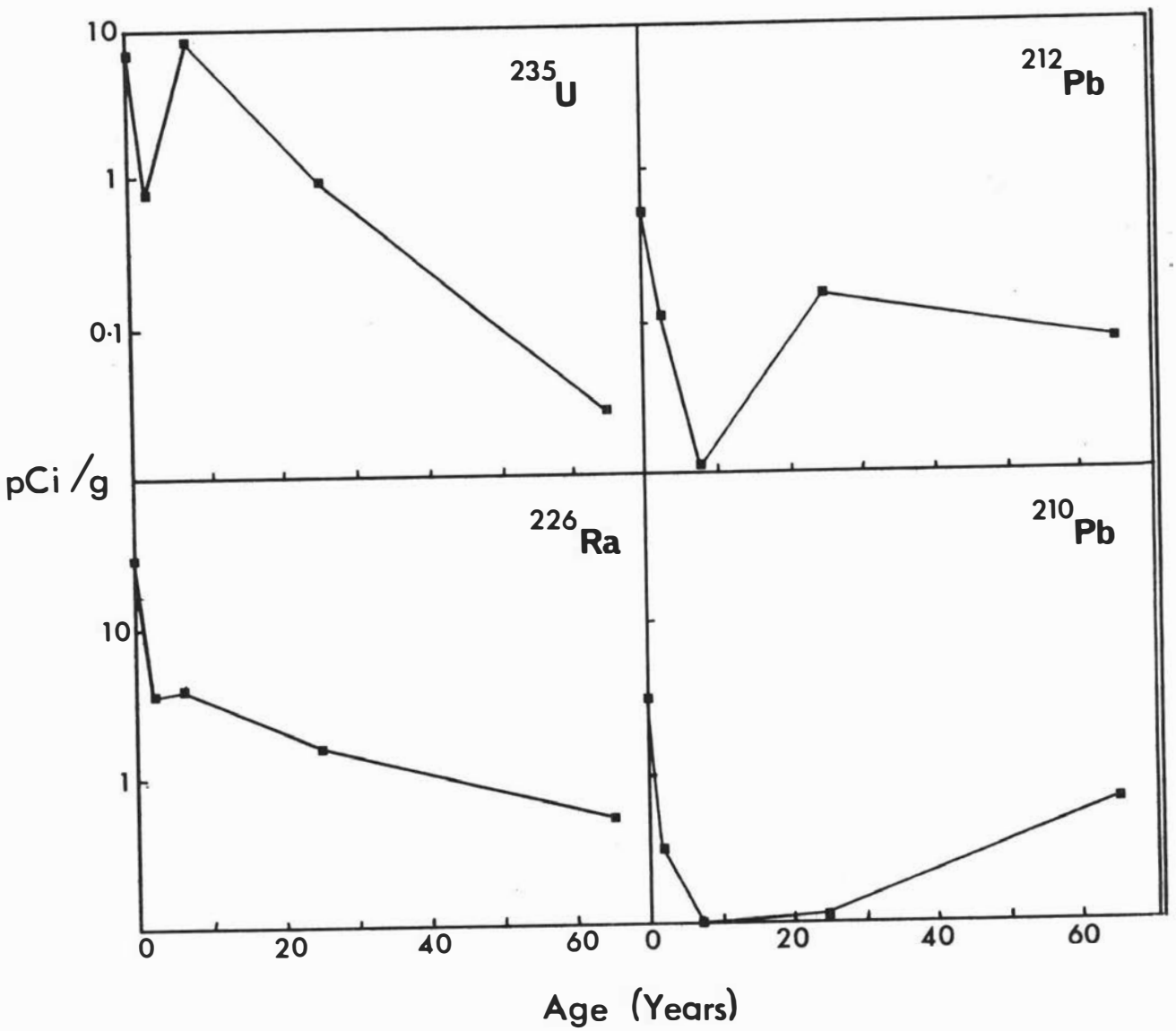
of fall-out on grass, by Potter et al., (1969), where the 277 keV peak was assigned to the isotopes ^{147}Nd and ^{239}Np, from the energy of emission, and from the fact that other peaks at 60, 91, 105, 116, 207 and 227 keV are known to correspond to the same isotopes. Since some of these are major emission energies of the isotopes concerned, and since they are not noticeably visible in fig. V.12., other isotopes must be responsible. After a period of some months, the innermost ring section was recounted, and the unusual peaks had all decayed, which put an upper limit on the half-life and suggested emission of ^{125m}Te, at 110 keV, ^{57}Co at 122 keV, and ^{129}Te at 277 keV.

These isotopes are certainly produced in fission (Gest and Glendenin, 1951; Marquez et al., 1959; Lowman, 1960), and both cobalt and tellurium are known to be absorbed by plants (Duvigneaud, 1959; Jacobson and Overstreet, 1948), but the assignations must remain in doubt since our present knowledge of the translocation of elements in tree-trunks across annual rings, is practically non-existent. The assignations are much more credible for the mosses, which are known to absorb many strange cations (Whitehead and Brooks, 1969).

Cooper et al., (1968) also claim that the 122 keV peak is due to ^{57}Co, but the only evidence they offer is energetic, and they did not observe the other unusual peaks.

3. Distribution of isotopes in annual rings

Little is known about the distribution of any elements in trunk sections, nor is it known what factors affect the absolute amount at any given time. For this particular tree, however, it seems likely that the normal accumulation of cations has been altered by mining operations near


10
1
0·1
pCi /g
235U
212Pb
226Ra
210Pb
10
1
0
20
40
60
0
20
40
60
Age (Years)

the tree in 1959, giving, as can be seen in fig. V.14., a high concentration ten years ago for ^{235}U and possibly for ^{226}Ra.

Generally the highest concentrations of radionuclides occur in actively-metabolising tissue, with a decrease towards the centre. The increase in the amount of ^{210}Pb probably reflects the increased amount of time available in the centre of the trunk for production from ^{226}Ra, but this factor of time is unlikely to be the explanation of the plateau observed in the ^{212}Pb figures. The radium ancestor of ^{212}Pb, is ^{228}Ra, with a half-life of only 6.7 years compared with 1602 years for ^{226}Ra. This means the activity is probably due to ^{232}Th which is also the suggestion of Gvozdanovic *et al.*, (1967), and Verkhovskaya *et al.*, (1967), in connection with old wood.

E. Discussion

1. Origin of observed isotopes

The samples to be counted were left to stand for 144 days in airtight containers to prevent radon loss, so many of the observed isotopes will be a direct measure of others. Inspection of fig. V.1., shows that ^{211}Bi, ^{212}Pb, and ^{214}Pb will be produced from various isotopes further up in the decay series.

The isotopes, ^{226}Ra and ^{235}U are already known to be absorbed from the soil rather than being produced in the plant from other sources. The average plant/soil ratios were 0.815 and 0.197 respectively. A plant/soil ratio could not be obtained for the mosses, or for the lichen Stereocaulon, because in both cases the cations are absorbed from water flowing past the plants, but as one would expect from its habitat, near a uraninite vein, the lichen contained a considerable amount of ^{235}U.

The ^{211}Bi could have originated from absorbed ^{223}Ra or even ^{227}Th, but a recount after three months of all samples containing a significant amount of ^{211}Bi showed no diminution in activity, and the presence of ^{211}Bi is therefore due to absorbed ^{227}Ac. This appears to be the first demonstration of the presence of this isotope in plant material. It is possible that a little ^{223}Ra with its half-life of 11 days was absorbed, but decayed before counting took place.

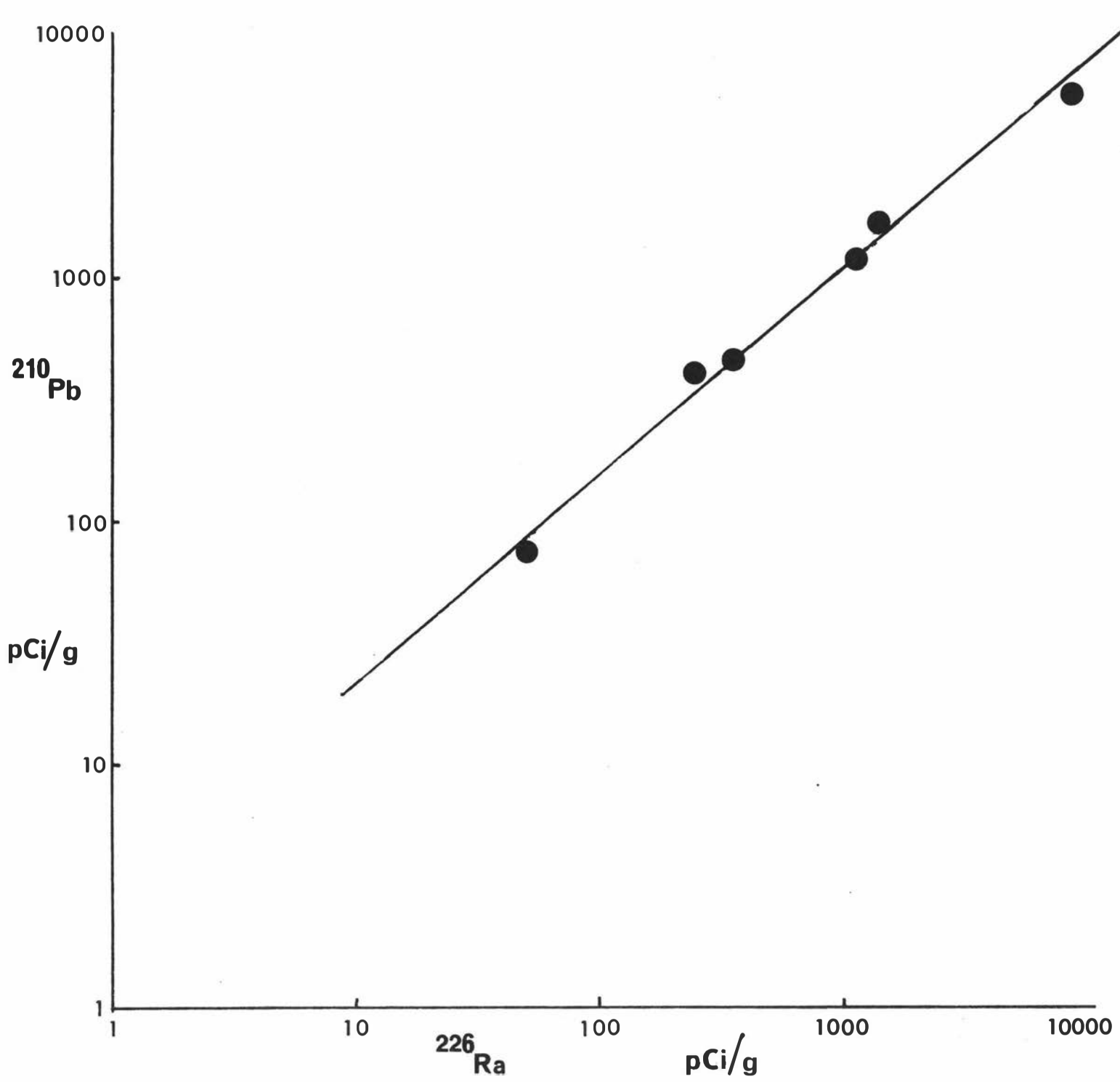
The ^{210}Pb in the samples could have originated from the decay of ^{226}Ra inside the plant, but even if it is assumed that the ^{226}Ra was all absorbed at the beginning of growth, ten years ago, calculation shows that the amounts of ^{210}Pb thus produced would be an order lower than the

observed values. The work of Tso et. al. (1966) already quoted, has shown that insignificant amounts of ^{222}Rn are absorbed from the air by plants, hence the absorption and decay of this isotope is not the means by which the large amounts of ^{210}Pb are produced.

There are two other possible sources for the ^{210}Pb; absorption from the soil, and fall-out. Fall-out has been already discussed in Section V.D.2. and can be eliminated as a source, leaving absorption as a mechanism of entry. The literature quoted earlier, suggested that lead is largely fixed at root level, and that little reaches the leaves, making this mechanism unlikely also. However Nicolas and Brooks (1969) have shown that in some New Zealand species substantial quantities of lead do reach the foliage, and analysis for total lead of three plants and their associated soils from the region under study, gave plant/soil ratios an order higher than those quoted as standard, by Wilson and Cline (1963). These high plant/soil ratios were more than sufficient to account for the observed levels of ^{210}Pb, and the conclusion must therefore be that the isotope is absorbed from soil, and that other contributions are minimal.

The associated soils were older than ten years, and it is possible that sufficient time had elapsed to produce the observed levels of ^{210}Pb from ^{226}Ra. This hypothesis is supported by the proportionality between ^{226}Ra and ^{210}Pb illustrated in fig. V.15.

The ^{212}Pb in the samples could originate from ^{228}Th, which fig. V.1. shows to be the first ^{212}Pb ancestor with a long enough half-life to be absorbed by plants. However, some or all of any ^{228}Th present, may be radiogenic, being produced from absorbed ^{228}Ra, decaying to ^{228}Th via


10000
1000
100
10
1
^{210}Pb
pCi/g
1
10
100
1000
10000
^{226}Ra
pCi/g

^{228}Ac. It is possible to gain some idea of the extent of this by calculation as follows.

From Table V.5. it is possible to calculate plant/soil ratios for ^{226}Ra, which must also apply to any other isotopes of radium, since they are chemically similar. Specifically, the ^{228}Ra plant/soil ratio must be equal to the ^{226}Ra ratio. If the ^{228}Ra content of the soil is known, then the ^{228}Ra content of the plant can also be calculated, and also the amount of ^{228}Th due to ^{228}Ra decay in any given time. Assuming approximate equilibrium in the soil, it can be seen that the soil ^{228}Ra content can be found from the ^{228}Th content, which in turn is given by the amount of ^{212}Pb. The amount of ^{228}Ra in the leaf is then calculated, and as in the case of ^{210}Pb, the extreme assumption is made that all the radium present in the leaf material was absorbed at the beginning of the plant's growth, hence ten years is allowed for its decay. This certainly is too long, but sets upper limits on the amount of ^{228}Th that could be produced.

The calculation yields the figures in Table V.6.

Table V.6. Comparison of calculated and observed values of ^{228}Th in various plants.

Plant	Calculated ^{228}Th	^{228}Th present in sample
B. procerum	0.24	37.2
C. australis	3.5	0.51
M. berteroana	negligible (not calculable)	11.7
N. fusca	not calculable, but very high	0.34
Q. acutifolia	" "	0.56
U. leptostachya	0.12	0.11
W. racemosa	0.67	0.06

Although the results may be equivocal for some of the higher plants, it seems clear that M. berteroana, and B. procerum absorb substantial amounts of ^{228}Th, and hence other thorium isotopes.

This accumulation of thorium isotopes is marked, since little trace of them can be found in the associated soils. Various peaks due to thorium isotopes are prominent in the spectrum of B. procerum, in fig. V.12., where one very unusual feature is the large amount of ^{230}Th. Very strong ^{227}Ac absorption is also suggested by numerous peaks, and is confirmed by the high ^{211}Bi plant/soil ratio. The 53 keV peak may be due to ^{234}U produced from ^{234}Th which has been absorbed and decayed, but the twin peaks at 195 and 200 keV could not be identified.

The spectra of the mosses did not indicate any consistent differences between mineralised and non-mineralised streams.

2. Isotopes responsible for alpha emission

Calculation of the alpha emission due to the various gamma-emitting isotopes and others in equilibrium with them is possible, if, as was possible in this case, absolute activities can be obtained.

If a radioactive decay series is in equilibrium, the rate of decay of one isotope equals that of the others in the chain. Similarly, unless branching occurs, the rate of emission of alpha-particles will be equal for all alpha-emitting isotopes. It is therefore possible to calculate the rate of alpha-emission by the whole chain, and compare this with other isotopes, and series of isotopes. For example, the contribution of the decay series between ^{226}Ra and ^{210}Pb to the alpha-count, will be four

times the ^{226}Ra activity, since there are four alpha-emitting isotopes in the sequence. However since the specimens for alpha counting were ashed just prior to counting, there will be no ^{222}Rn present, and the total activity will be only three times that of ^{226}Ra.

The contribution from ^{210}Pb may be expected to be negligible, since it depends entirely on the decay of ^{210}Po, a daughter, which was also removed by ashing, and again there is not enough time for a significant amount of ^{210}Po to be produced. Even apart from this, absorbed ^{210}Pb is relatively low.

The contribution of each isotope and descendents in equilibrium with it to the total alpha-count, was calculated, and the results are shown in Table V.5.

A major part of the alpha-activity is due to the disintegration of ^{238}U. It has been calculated (Coote, Cohen and Whitehead, 1970) that the activity of this isotope is 21.7 times that of the very much rarer ^{235}U, which however has a shorter half-life.

Table V.7. gives the means of the various percentage contributions to the total alpha activity. By this table, the prospecting method of Anderson and Kurtz (1955) is shown to be justified. This method involved counting of plant ash and taking the resultant alpha figure as a measure of the uranium in the soil. Not only does the table show that most of the activity in the plants is due to uranium isotopes, but also that the major activity in the soil is contributed by the same isotopes. The validity of this method should hold unless an area rich in the thorium decay series is encountered (which will be obvious from the gamma spectrum), or

Table V.7. Percentage contributed to the total alpha count by various isotopes and others in equilibrium with them.

	^{226}Ra	^{211}Bi	^{235}U	^{212}Pb	^{238}U
Plant	14.3%	7.6%	3.4%	0.8%	73.9%
Soil	14.1%	6.5%	4.6%	6.8%	68.0%

material from a radioactive anomaly, which contains radium, but almost no uranium, is examined.

In summary, this investigation has succeeded in identifying in a manner rather more rigorous than usually employed, the gamma-ray spectral peaks emitted by plants and soils taken from a uraniferous area. These findings have been used to yield information about the uptake of various radioelements.

For the first time, the absorption of ^{227}Ac by plants has been shown. This was especially evident in the lower plants studied, which also contained substantial amounts of various thorium isotopes. Many plants absorbed lead, and the findings described suggest that in New Zealand many plants have a greater ability to absorb this element, than has been generally reported elsewhere. This suggests that biogeochemical prospecting for lead will probably be simpler here from an analytical standpoint.

From the gamma-ray activity it was possible to calculate the alpha-activity, and show that alpha-activity measurements on leaf ash will generally be a good measure of the uranium content. In passing it should be noted that the beta-activity could also be calculated from the gamma-ray figures, should this be required for any reason.

These results show clearly the versatility of the method, which because of the lack of sample preparation, and its non-destructive nature, is very attractive. The equipment, especially the germanium detector, is not cheap, but the cost may be expected to fall as the potential and application of the method become more widely known.

SECTION VI

THE PLANT BIOCHEMISTRY

OF URANIUM

A. Introduction

At present in biogeochemical work, it is necessary to carry out an orientation survey, to determine whether or not a given plant species sampled in the test area, will contain an amount of an element bearing a definite relationship to the amount in the soil or bedrock. Orientation surveys are important, because there are a large number of variables influencing the uptake of elements by the plants, and the relative importance of these is not yet sufficiently well-known to enable prediction of the usefulness of a plant to be successful. Section V. shows that one of the important variables affecting the uptake of an element, is the physiological peculiarities of the plant itself. It was shown that there were differences in the uptake of thorium isotopes by various plants, and that for some reason, the fern Blechnum procerum was capable of absorbing many times more thorium isotopes than other plants although the soil conditions were substantially the same. The fern must have some physiological mechanism for concentration of thorium which other plants lack. In contrast, the absorption of radium and uranium was high for most plants.

If it were possible to predict which type of plant was an accumulator, much time could be saved in biogeochemical surveys, and this could be specially useful for those elements which are usually excluded from the plant.

For a plant to accumulate large amounts of a trace element, protective mechanisms may be necessary. Many trace elements act as co-factors in enzymic reactions, and an unbalancedly high rate for one or more reactions may lead to gross abnormalities of metabolism. One simple protective device, is chelation. This, if the chelation is reasonably efficient, effectively removes the cation in question from general cell reactions. Possible chelating

agents are the various biopolymers, and the organic acids and bases which are intimately bound up in the metabolism of the cell.

According to Poole and Poel (1965), cation uptake in general is balanced by organic acid synthesis, hence if a cell is capable of producing large amounts of organic acids, it may well be a potential accumulator of various elements. Such cells would inevitably have a rather low cytoplasmic pH, and it might be possible to determine whether an individual plant was or was not an accumulator for an element, simply by the use of crushed leaves, and universal indicator paper. This theoretical prediction is borne out to some extent by Cannon (1957), who found that uranium is best absorbed by plants with a fairly acidic cell sap.

None of the plants studied in the previous section concentrated uranium spectacularly except the mosses, but it seemed worthwhile to study the chemical form of uranium in a common shrub, Coprosma australis, in the hope of gaining clues as to what might be biochemical prerequisites for uranium accumulation.

Uranium has long been known to have effects of a profound nature on various plants. Up to a certain very low concentration of uranium, these effects are stimulatory (Loew, 1904; Chirikov, 1913; Drobkov, 1939; Drobkov, 1949.) Higher concentrations decrease the yields, and one explanation offered (Acqua, 1913) is that the uranium specifically interferes with cell division, by combining with what is called in modern terminology, the nucleic acids. As regards the actual chemical form of uranium in plants, the only information appears to be from work on yeast.

Rothstein and Larrabee (1948), showed that uranium did not significantly penetrate into the yeast cell, being bound on to the cell membrane as a complex with a stability constant of 5×10^6. (Rothstein et. al., 1948). A second type of binding was also identified, possessing weaker complexing powers for uranium, and the two types were tentatively ascribed to phosphate and carboxyl groups respectively. In retrospect it seems quite possible that the two sites are nucleic acids and proteins respectively, especially since later work showed that the properties of the main binding site closely resembled the chemical behaviour of either polyphosphates or nucleic acids in response to stability of binding of uranium under the influence of pH, and other variables. (Rothstein and Meier 1951).

It is also known from work on animals, that uranium combines with the cell membranes, and interferes with the free passage of various substances especially glucose (Singer et. al., 1947). Unfortunately no chemical studies were done on the nature of the binding site.

It is not certain how far these results may be extrapolated for higher plants. If all uranium is adsorbed on to cell walls, then we would expect none to penetrate beyond the level of the roots, but the fact that some is found in the leaves, suggests that further examination is needed. This examination will need to start from a more basic level, so that some of the chemistry of uranium is now reviewed briefly.

Uranium has two common valencies, +6 and +4, though the former is more common, unless the conditions are strongly reducing. Solutions containing the U^{+6} ion are strongly hydrolysed, and the uranium exists in the form of the uranyl ion UO_2^{+2}. If the pH of such an acidic solution is adjusted

to neutrality, it is usual to obtain a precipitate of a uranate. Such precipitates are not obtained in living cells, because many cations are capable of chelating the uranyl ion. Table VI.1. gives a list of complexes of uranium of possible physiological interest together with an indication of relative stability.

Two classes of molecules which are found in living cells and do not significantly chelate uranium are the lipids, and most of the carbohydrates, neither of which possess the correct functional groups (Hurwitz and Rothstein, 1951; Muntz et al., 1947).

An important feature of work described in the literature is the ability of proteins to chelate uranium. This was first noticed in connection with enzyme studies. The following are some of the enzymes, whose action is known to be inhibited by the action of uranium: ptyalin and maltase (Roger, 1909), takadiastase, catalase, serum and liver lipase (Mikawa, 1924), invertase (Lardy and Anderson, 1942), urease, lysozyme, hexokinase, glucose-6-phosphate oxidase, succinoxidase (Singer et al., 1947), phosphomonoesterase (Castella Bertran and Rife Bertran, 1950).

Unlike much enzymic inhibition due to heavy metals, the action of uranium does not depend on combination with free sulfhydryl groups, since these groups can still be titrated with specific reagents once the uranium is attached to the protein. The workers in this area concluded that the manner of attachment was quite different, and attempted to reverse the inhibition by using various known chelators of the uranyl ion, to remove the uranium from the protein. It was thought that some idea of the type of binding site might be gained (Singer et al., 1947).

Table VI.1.

Stability constants for selected uranyl complexes

Ligand	K_1	K_2	K_3	References
Carbonate	-	4×10^{14}	2×10^{18}	Li et al. (1957)
Oxalate	4×10^{6}	1.2×10^{11}	-	Talipov et al. (1961) Stary (1960)
Nucleic acids	-	-	1.4×10^{7}	Zobel and Beer (1961)
∝-OH butyrate	3.8×10^{3}	2×10^{5}	10^{7}	Stary and Balek (1962)
Citrate	3.4×10^{3}	-	-	Li et al. (1957)
Protein	-	-	2×10^{6}	Rothstein and Larrabee (1948)
Acetate	4×10^{2}	8×10^{4}	2.2×10^{6}	Feldman and Koval (1962) Stary (1960) Ahrland (1951a)
Salicylate	-	-	1.24×10^{5}	Kumar Dutt and Goswami (1959)
Glycolate	5.5×10^{2}	1.2×10^{4}	3×10^{5}	Stary and Balek (1962)
Lactate	6.4×10^{2}	3.6×10^{4}	2.8×10^{5}	" "
Glutamate	4×10^{2}	-	-	Feldman and Koval (1962)
Succinate	4×10^{2}	-	-	" "
Sulphate	-	3.5×10^{2}	-	Ahrland (1951b)
Malate	1.8×10^{2}	-	-	Li et al. (1957)
Phosphate	15.5	21.8	-	Thamer (1956)

Monocarboxylic acids proved ineffective, but some dicarboxylic acids were quite powerful in reversing the inhibition. The structural criteria for this appeared to be the presence in the molecule of a hydroxyl group and two carboxyl groups, since citric acid was quite a good chelator, but tricarballylic acid, which lacks the hydroxyl group of citric acid, was of no effect. The most powerful reverser of inhibition was hydroxyaspartic acid which is an acidic amino acid. From these results it seems likely that the binding of uranium to proteins, is mediated through the carboxyl groups with the co-operation of hydroxyl groups from other amino acids such as leucine, and that the strength of this binding is slightly less than the dicarboxylic acids detailed in table VI.1..

Besides enzymes, it is known that the blood proteins chelate uranium (Guzman-Barron, 1951; Muntz and Guzman-Barron, 1951), and this binding can also be reversed by such chelators as citrate and bicarbonate. It will be remembered that Glover (1953) made this affinity the basis of a separation procedure in the assay of uranium.

Nucleic acids are even more superior in the chelation of uranium. Various uranyl salts have been used for some time as a stain for ribonucleic acid (RNA) in microsomes and phage particles (Valentine, 1958; Huxley and Zubay, 1960), following the observation of Rothstein and Meier (1951) of their selective staining abilities. The most significant paper on this subject is by Zobel and Beer (1961) who were interested in using uranyl salts as a selective stain for nucleic acids in electron microscopy. Their careful work showed that the binding of uranium to deoxyribonucleic acid (DNA) was almost certainly through the phosphate groups rather than the bases on the inside of the spiral. The evidence for this was that, firstly, studies of Von Wazer and Campanella, (1950)

and Rothstein and Meier (1951) had shown that that amount of uranium bound to nucleic acids and high-molecular-weight polymers of phosphate, was in each case exactly proportional to the number of phosphate groups available for binding, secondly that Zobel and Beer (1961) showed from infrared studies that the only spectral changes in DNA spectra when uranium was bound, occurred in peaks thought to be associated with the phosphate groups. Thirdly, Beer and Zobel (1961) bound uranium on to DNA and examined it under the electron microscope at such a high magnification that individual uranium atoms were visible. The small electron-opaque dots thus observed were 20 Å apart which is what one would expect if they were bound to the adjacent pairs of phosphate groups rather than the bases (this is one of the very rare occasions when evidence for chemical structure has been obtained by direct observation).

All the above work showed that there were quite a number of different molecules in the cell which have a relatively high affinity for uranium, but some cast doubt on whether uranium actually penetrated past the cell membrane, to come in contact with those molecules. None of these detailed chemical studies had used material from higher plants, and research in this area was certainly needed. The research described in this section was done in an effort to satisfy curiosity as to how a higher plant survives the toxic effects of uranium, and, since it was guessed that this would involve chelation in some form, to see whether the findings gave any indication of possible properties, which would render a plant a likely accumulator of uranium and useful in biogeochemistry.

B. Preliminary fractionation of tissue

Leaves of Coprosma australis from the previously described mineralised area, were freeze dried to give a total of 100 g of dry material. This was ground in a hammer mill to pass a 0.5 mm metal screen, and stored out of the light completely enclosed in plastic. The powder proved to contain 6 ppm uranium (dry-weight basis).

1. Method

The solvent fractionation methods of Bowen et. al. (1962) was followed, to divide the tissues into three fractions.

Three grams of freeze-dry tissue were refluxed in 250 ml of 70% ethanol for 20 min. The suspension was filtered using a Buchner funnel, and the insoluble material refluxed in distilled water for a further 20 min. Filtration of this suspension yielded a grey-brown residue. The three fractions, ethanol-soluble material, water-soluble material and residue, were each analysed for uranium. Analyses were in quadruplicate; all other analyses reported in this section are at least in duplicate.

2. Results

The percentage uranium occurring in each fraction is given in table VI.2.

The ethanolic fraction normally contains simple ions, low molecular weight complexes of some polarity, and non-polar pigments. In this case the uranium recovered in this fraction was minimal, which suggested that the uranium was not present as a simple uranyl ion, or attached to easily-removed non-polar material. This result suggests chelation.

Table VI.2.

Percentage distribution of uranium in different chemical fractions of freeze-dried C.australis leaves

	Treatment		
	70% ethanol	water	extracted residue
Batch 1	8	72	20
Batch 2	5	67	28

The aqueous fraction contained the bulk of the uranium, so the complex of uranium is rather polar.

The insoluble residue contained a moderate amount of the total uranium, and the element was probably bound moderately strongly since the reflux conditions used were rather vigorous, and probably loosened the tissue structure to such an extent, that the solvent was able to come in contact with most parts of it.

C. Investigation of the water-soluble material

1. Electrophoresis

Electrophoresis sorts material according to charge, and can give information about the charge of the uranyl complex, and indirectly, some of the possible ligands.

(a) methods

The aqueous extracts from a number of extractions were combined and evaporated to low volume under vacuum. The solution became dark, and precipitated a dark-brown solid. This was isolated by centrifugation at 26,000 g for 15 min. and analysed. It contained less than 5% of the uranium in the aqueous fraction, so was discarded, and the centrifugation used as a routine step. Preliminary experiments showed that the maximum possible loading of the paper was with an extract containing the equivalent of 3 g of original freeze-dry material.

The paper was Whatmans No.1 chromatography paper cut to a size of 43 x 53 cm and prewashed for a week with 0.1 M pyridine-acetate buffer, (pH 5.3) and many changes of distilled water. The sheets of paper were once again soaked in buffer, and the appropriate amount of extract was streaked vertically across, following which the sample was electrophoresed at 300 mA and 5.5 kV for 5 min. in a Miles Hivolt electrophoresis apparatus. The buffer was then removed by volatilisation in a current of warm air.

Various spray reagents, like ferrocyanide, were used in an attempt to localise the position of the bands containing uranium but this did not prove to be possible partly because the amounts present were so small, and partly because the complex in the extract was very probably more stable than the

ferrocyanide complex itself. It was necessary to divide the paper into vertical 12 mm strips and analyse each for uranium.

To establish the charge on a given band, markers of known charge were run alongside the sample. These were mainly various uranium complexes synthesised as follows; A solution of uranyl nitrate was treated with hydrogen peroxide to precipitate a complex yellow uranium oxide, which was filtered off and dissolved in a small excess of sodium carbonate solution. The resultant complex is uranyl tricarbonate, three being the maximum co-ordination number of doubly charged anions for uranium. This dark brown solution was not crystallised, but portions were taken and treated with excesses of various common acids to form the appropriate complexes, when required, no complex being isolated except those which spontaneously crystallised.

The complexes prepared were the tricarbonate, tricitrate, trimalate, triacetate, tritartrate, trioxalate, trinitrate, trisuccinate, triglutamate, triaspartate, and trisalicylate, which are some of the possible forms uranium might take under physiological conditions. All these complexes have either a charge of -1 or -2. As a neutral marker it was necessary to use leucine, an amino acid, since there are few readily accessible complexes of uranium which are uncharged. Sufficient of a given marker was used to enable detection to be made by the spray reagent ferrocyanide, or in the case of the amino acid, a ninhydrin spray reagent.

(b) Results

The electrophoretograms were examined visually before analysis. In normal light, a brown band was seen spreading to the positive side of the origin. Under ultraviolet light at either 254 or 350 nm, the region immediately

beyond this band contained some compound which absorbed the light strongly (see Fig. VI.1.). Analysis showed that it was this region which contained the uranium complex.

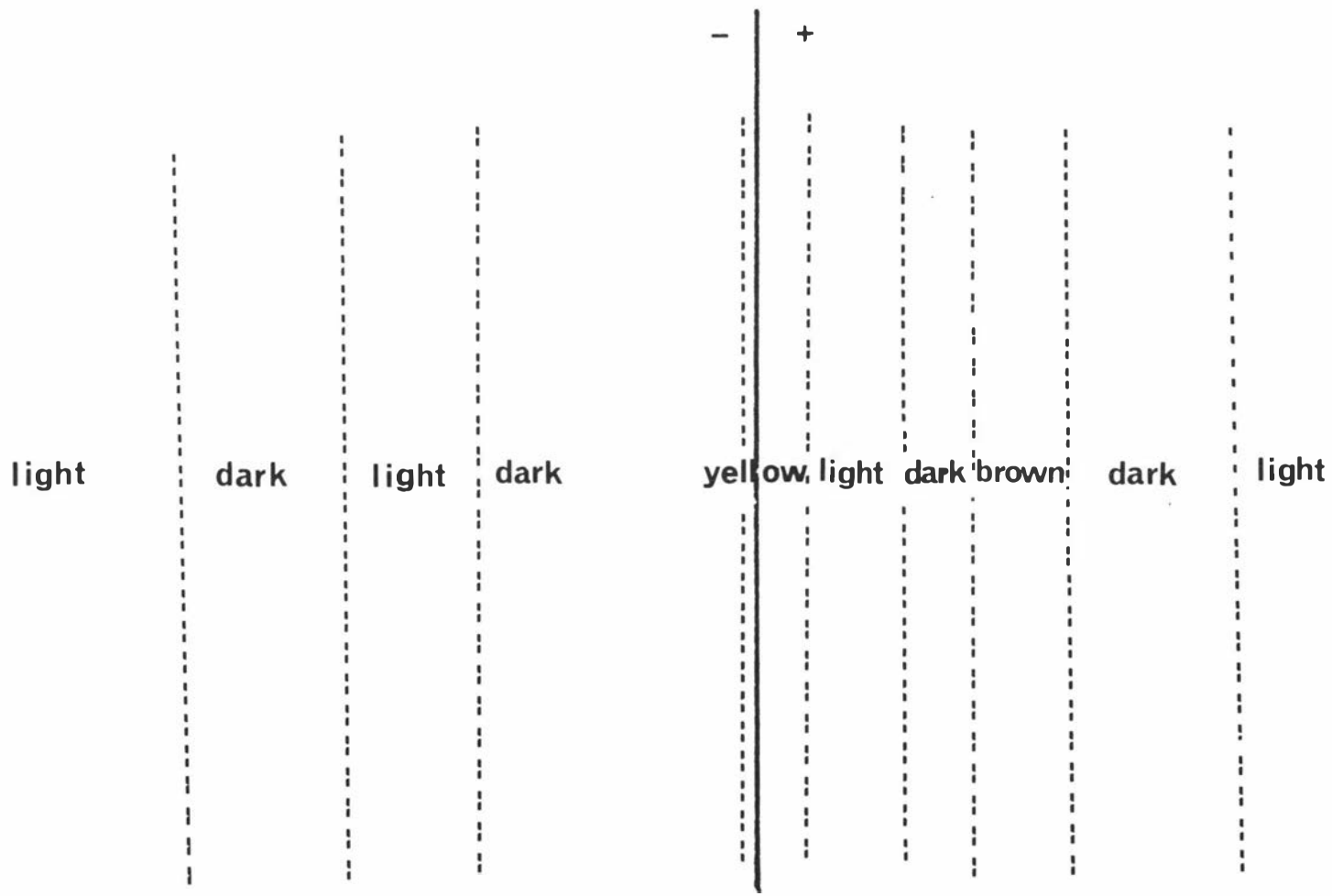
The combined results from analyses of two electrophoretograms are shown in fig. VI.2. The major part of the uranium travelled much further than any of the markers, and was therefore not the same as any of them, but this failure to synthesise a marker with a comparable charge, also means that the charge on the unknown could only be estimated, and was probably between -3 and -4. This is surprisingly high, and suggests that the ligand was either a very unusual compound of low molecular weight, or a macro-molecule like a protein or nucleic acid.

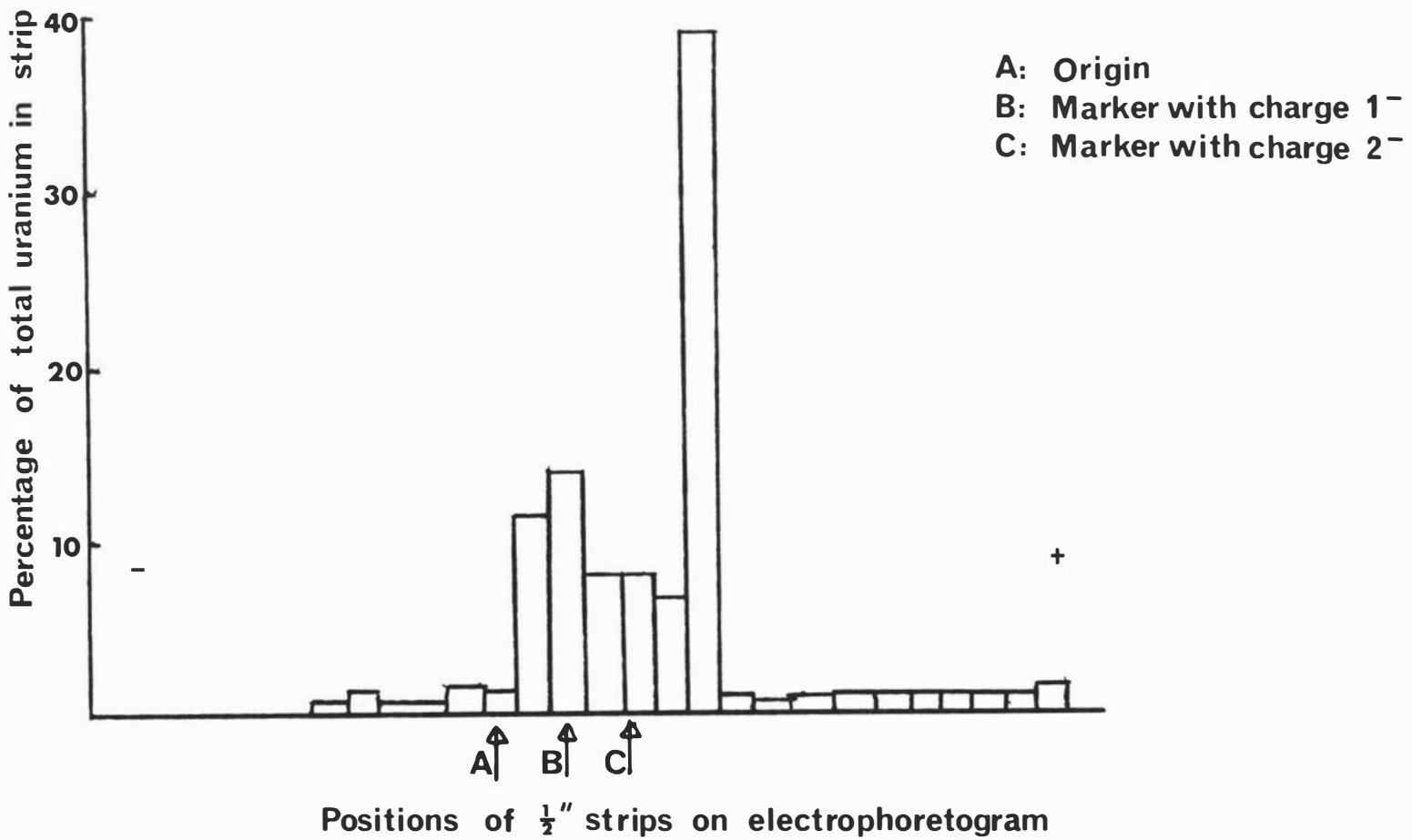
2. Molecular weight determinations

(a) methods

The appropriate strip from the electrophoretogram was eluted twice with a small volume of distilled water. Analysis showed that this treatment removed more than 99% of the uranium. The eluate had a pH of 3 and was only very faintly coloured compared with the original implying a good degree of purification. It was concentrated *in vacuo*, and ultra-filtered.

The apparatus used was a Diaflo No. 50 ultrafiltration unit, manufactured by the Amicon Corporation. Liquid is forced through special membranes under a pressure of 3.5 kg/cm^2 of nitrogen, the membranes being constructed so that only molecules of a given molecular weight can penetrate. If a range of membranes is used, it is possible to delimit the range in which the molecular weight of the sample falls. The membranes used were able to pass the following maximum molecular weights: (a) 100,000; (b) 20,000;


–
+
light
dark
light
dark
yellow
light
dark
brown
dark
light


Percentage of total uranium in strip
40
30
20
10
-
+
A
B
C
A: Origin
B: Marker with charge 1⁻
C: Marker with charge 2⁻
Positions of ½" strips on electrophoretogram

(c) 10,000; (d) 1,000. These were used sequentially and the resulting five fractions were analysed for uranium.

(b) results

Rather erratic results were obtained, and the results of two typical estimations are given in table VI.3. The only difference between the two estimations was that the first solution had been prepared and tested immediately, whereas the second had been left to stand at room temperature for a week.

The only conclusion possible is that the material is heterogeneous or is a biopolymer exceptionally labile on standing in acid. Making a rough extrapolation backwards it seems likely that most of the fresh material had a molecular weight greater than 20,000 d.

3. Infrared and ultraviolet spectra

An infrared spectrum of the compound was not satisfactory. The peaks were too broad for unequivocal identification of any functional group.

The ultraviolet spectrum was obtained using a Unicam SP800 spectrophotometer. This is a double-beam instrument and the blank was an eluate from paper which had been washed in the same way as the electrophoresis paper, but not been subjected to electrophoresis with a sample.

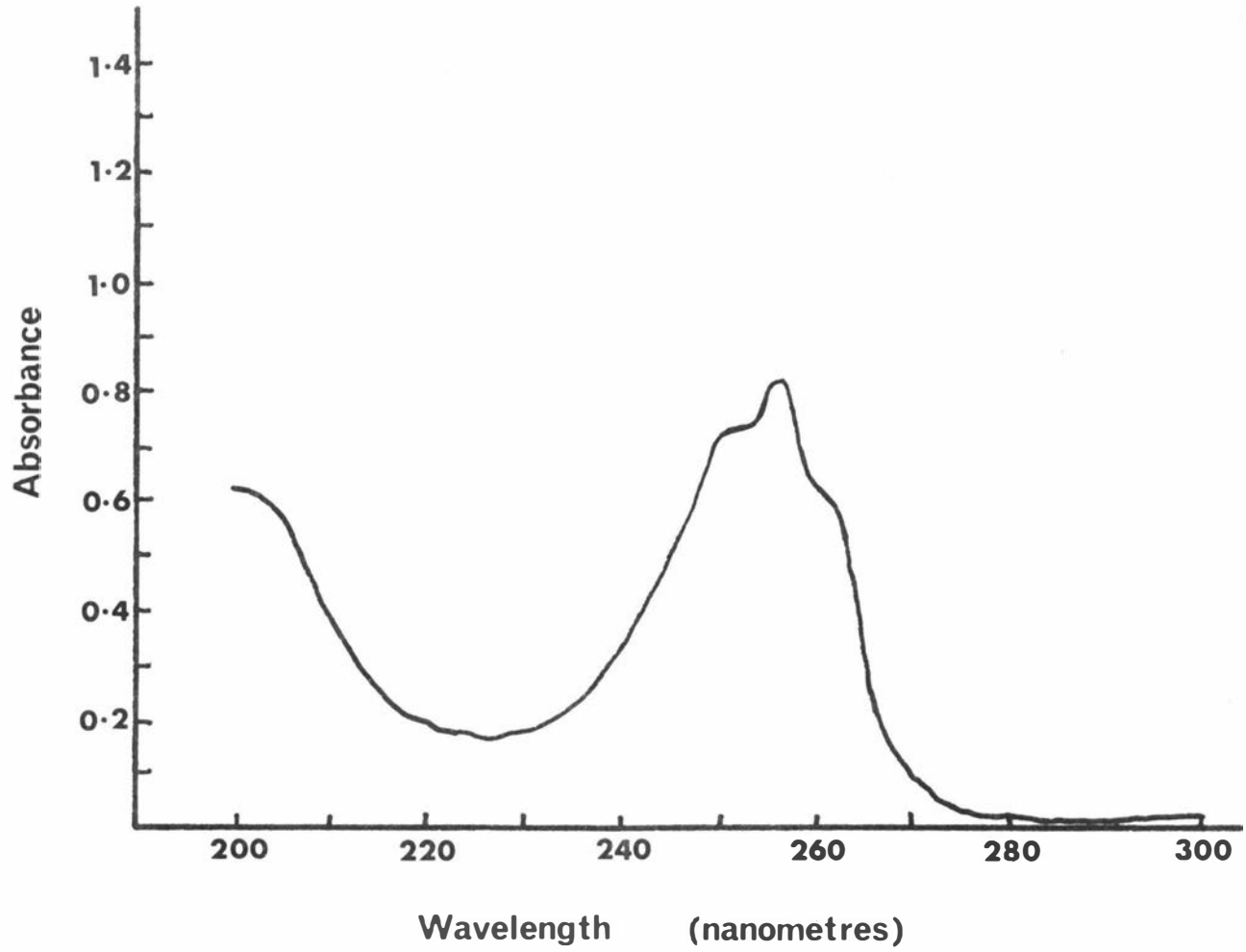
The spectrum is given in fig. VI.3. There is very little absorption at 280 nm which would indicate the present of protein, but substantial absorption at 256, which probably indicates the presence of nucleic acids or their component bases. However this was circumstantial evidence only,

Table VI.3.

The distribution of uranium in different molecular weight fractions of a C.australis extract.

Fraction*	2 days preparation only	7 days storage
100,000d	22	18
20,000 - 100,000	48	2
10,000 - 20,000	0	1
1,000 - 10,000	30	66
1,000	0	13

*Molecular weights represent uranium compounds fractionated by the serial use of Diaflo filtration membranes.


Absorbance
1·4
1·2
1·0
0·8
0·6
0·4
0·2
200
220
240
260
280
300
Wavelength (nanometres)

and even if the substances causing the absorption were nucleic acids there was little evidence that the uranium was bound to them.

4. Tests for nucleic acids

(a) liberation of phosphate

Free phosphate cannot be detected in nucleic acids, but is liberated on hydrolysis. The compound suspected to be nucleic acid was hence hydrolysed and tested.

The qualitative method of Feigl (1967) for detection of free phosphate depends on the formation of a phosphomolybdate complex which reacts with benzidine to give an intense blue coloration. The electrophoretogram was sprayed with a solution of 0.05% benzidine in 10% acetic acid, and 3.4% ammonium molybdate in 1:4 nitric acid, and exposed to ammonia vapour. A blue coloration was seen on the positive side of the origin, stopping just short of the location of the suspected nucleic acid.

A sample of 0.5ml eluted compound was hydrolysed with 1 ml of 3 M nitric acid for one hour at 100^{o}C, and 50 mg of ammonium molybdate and 1 ml of 0.05% benzidine in 10% acetic acid was added. On addition of 0.2 ml of concentrated ammonia to the test solution and a suitable unhydrolysed blank, a deep blue coloration developed in the test solution compared with the blank showing the presence of a compound that releases phosphate on hydrolysis.

(b) DNA

The indole reaction of Ceriotti (1952) is specific for the sugar moiety of DNA and highly sensitive. Two ml of solution to be tested were mixed with 2 ml of 0.02% indole in concentrated HCl, and put in a boiling

water-bath for 10 min. After cooling the solution was extracted three times with 4 ml of $CHCl_3$ to remove various coloured substances which may form and interfere with the final measurement. The optical density of the aqueous solution at 490 nm was measured with a Unicam SP800 spectrophotometer.

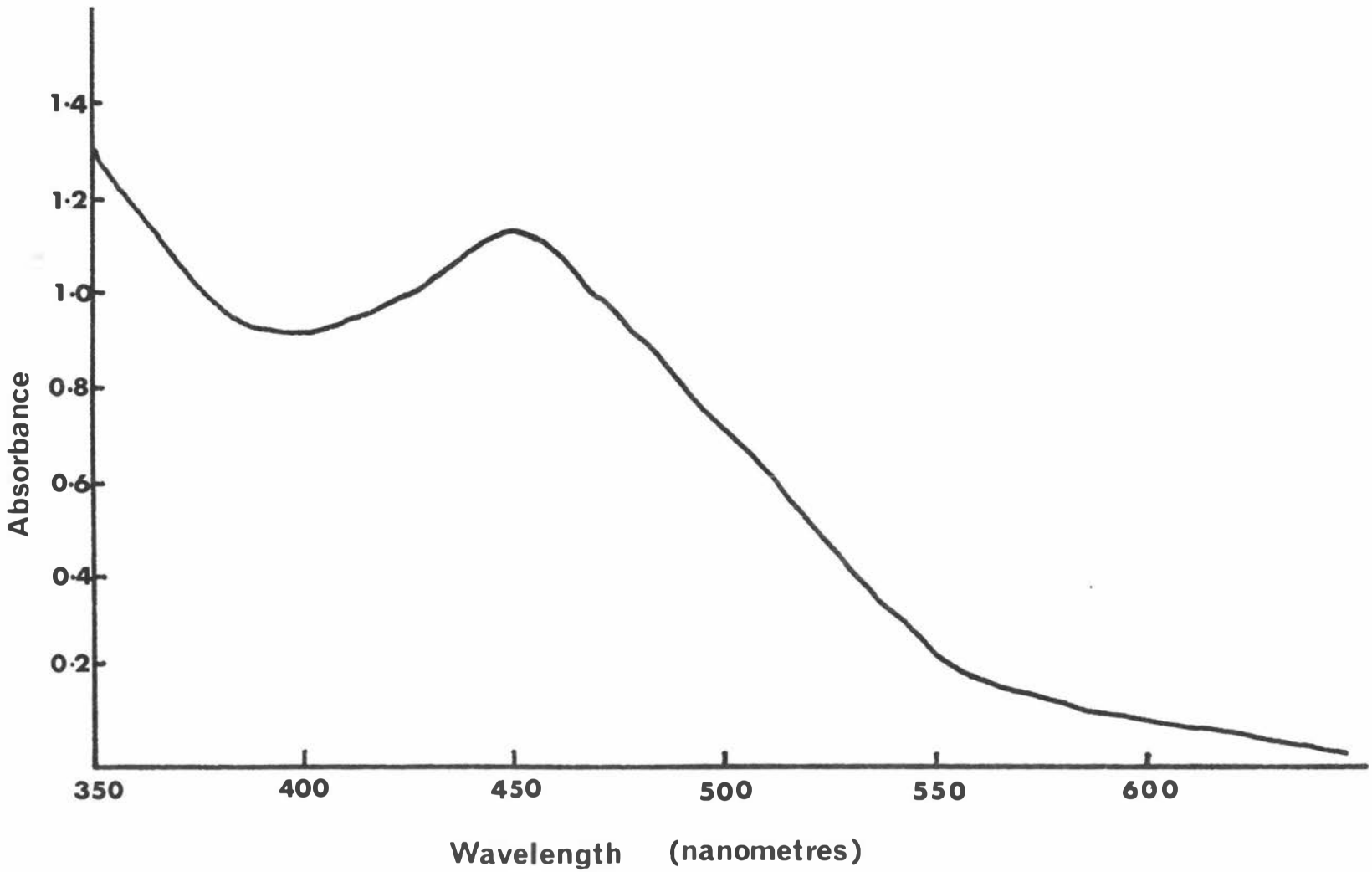
During the period of heating on the water-bath, a pink colour developed which extracted completely into chloroform, showing that there was some impurity present. The aqueous solution itself had less than 0.02 optical density units of absorption at the stated wavelength showing that there was no significant concentration of DNA in the sample.

The substances giving the pink colour observed are stated by Ceriotti (1952) to be RNA, ribose, levulose, glucose and other sugars, which suggested that although DNA was not present, RNA might be.

(c) RNA

Qualitative estimation of RNA was according to the method given by Svennerholm, (1957).

Two ml of a 2% resorcinol solution in concentrated HCl, 0.00001 M with respect to cupric ions, was added to 2 ml of unknown solution (eluate from the electrophoretogram) and heated in a boiling water-bath for 15 min. After cooling, 4 ml of amyl alcohol was added and the tubes shaken. The spectrum of the amyl alcohol was obtained on the Unicam SP800, and is depicted in fig. VI.4. There is little significant absorption at 580 nm, which means that the peak is due to ribose derived from RNA, with little interference from other compounds such as glucuronic acid.


Absorbance
1·4
1·2
1·0
0·8
0·6
0·4
0·2
350
400
450
500
550
600
Wavelength (nanometres)

It was concluded that RNA is present, but these experiments left open the question of whether the uranium was actually bound to it, or to some other substance. To approach this problem it was necessary to discover the conditions under which the uranium became freed from the polymer.

5. Enzymic hydrolysis

At this point enzymic hydrolysis seemed attractive. In theory it should be possible to determine whether uranium was attached to any given biopolymer by incubating the sample with an enzyme specific for that polymer, and measuring the amount of uranium passing through the ultrafiltration membrane. If the correct enzyme were chosen it should be possible to reduce very considerably the molecular weight of the chelator. This procedure was tried with DNAse, RNAse, and pepsin, which degrade DNA, RNA and protein respectively, but in no case was uranium observed in the ultrafiltrate. In view of later results, this is probably due to uranium being released by enzymic action, but then strongly chelated by the enzyme protein itself, and hence still being unable to pass the ultrafiltration membrane.

6. Chemical hydrolysis

Chemical methods of hydrolysis are not subject to the limitations of enzymic hydrolysis. They do not produce complexes too large to pass through the ultrafiltration membrane, and the hydrolysis is usually complete if continued long enough. A literature search showed that RNA, DNA and protein are hydrolysed under conditions sufficiently different to enable a selective and sequential degradation to proceed.

RNA is an exceptionally labile species, being completely hydrolysed if exposed to M perchloric acid for 24 hours at $4^{\circ}C$, and DNA requires

0.5 M perchloric acid, for 40 min. at 70°C. Proteins are relatively stable, requiring refluxing with 3 M sulphuric acid for at least twelve hours. A similar alkaline degradation is possible but requires much more vigorous conditions, and in practice it was found that the silicic acid dissolved from the glass interfered with the uranium estimations.

(a) method

A sample of eluate was made molar with respect to perchloric acid and allowed to stand for 24 hours at 4°C. The solution was ultrafiltered through a membrane passing only substances less than 1,000 dalton. The residue was incubated at 70°C for 40 min., without changing the molarity of the acid, and again ultrafiltered. All three fractions were analysed.

(b) results

All the uranium (within experimental error) was found in the ultrafiltrate from the step which hydrolysed RNA. When this is compared with the distribution of uranium obtained on the rough molecular weight estimations attempted in part C.2 of this section, a dramatic reduction in molecular size is noted, and this seems open to only one interpretation, that all the uranium in the aqueous fraction is bound to RNA. There is no other known biopolymer that degrades under these exceptionally mild conditions.

7. Discussion

The only compound unequivocally identified as occurring in the electrophoresis strip with the uranium was RNA. The chelating ability of this molecule towards uranium is so high that normally any uranium present in a solution would be found attached to it, but proof of this depended on the other experiments described.

Specifically, the molecular weight determinations and chemical hydrolyses showed that the uranium was bound to a biopolymer that is exceptionally labile to acid, and the only known compound that fulfills these requirements is RNA. It is concluded that in the aqueous fraction, uranium is chelated by RNA, with very little, if any contribution by any other substance.

D. Investigation of insoluble material.

1. Incubation with pepsin

It seemed reasonable to propose that the uranium might exist in two types of binding, one in the residue and another in the aqueous fraction, since most of the nucleic acids were quite possibly removed in aqueous extraction. Another obvious candidate for a chelation site was protein, so the residue was tested to determine whether a significant amount of the uranium was bound in this fashion.

(a) method

One gram of insoluble residue was gently shaken with 40 ml of 0.5% 1:2500 BDH pepsin in 0.1 M HCl. After 24 hours at 37°C, the soluble material was separated from the insoluble and both fractions were analysed for uranium.

(b) results

All the uranium was found in the soluble portion, which showed that pepsin could release uranium from the type of binding found in the residue, and showed that this binding was therefore to protein.

2. Chelating ability of some components of the residue

Thus far it was established that after extraction of tissues with ethanol and water, the uranium was found to be attached to nucleic acids and proteins. The next experiment was an attempt to show if any other types of compound were capable of binding large quantities of uranium.

(a) method

Nucleic acids were completely removed from some fresh freeze-dry

material by the incubation of freeze-dry material with 0.5 M perchloric acid at 70°C for 40 min. (Ogur and Rosen, 1950), a treatment which Wannemacher et al. (1965) have shown does not remove protein.

The protein was then removed by shaking 1 g of material with 100 ml of 0.01 M tris HCl buffer, pH 7.4, for 40 hours at 37°C, and filtering, to give a residue which probably contained mainly lipid and polysaccharide (it was established by a parallel incubation with pepsin that this treatment does indeed extract protein). To the protein fraction, and the final residue were added 400 micrograms of uranyl nitrate, and both fractions were extensively washed with distilled water by ultrafiltration, or exhaustive dialysis, as appropriate. Each fraction was then analysed to measure its relative binding capacity for uranyl ions.

(b) results

The uranium bound to the protein was equivalent to 1% of the weight of the latter, but within experimental error, no binding at all could be detected for the final lipid-polysaccharide residue. It is interesting that a sample of pepsin also gave a figure of 1% for binding ability. Qualitative acidification of the protein fraction reduced its ability to bind uranium by 87%.

This shows, first of all, that the treatment with buffer actually did remove all significant protein and also, that freeze-dry tissue from which nucleic acids and proteins has been extracted does not possess measurable uranium-binding properties and that no other components formed bonds with uranium except proteins and nucleic acids.

E. Investigation of unfractionated tissue.

1. Extraction of uranium by nucleic acid fragments

(a) methods

The literature already cited, suggests that nucleic acids are much better than proteins as chelating agents. If this is true, however, there exists the possibility of transfer from protein to nucleic acid during extraction procedures and possibly a very small amount of transfer in the reverse direction. During the extractions described, a large amount of uranium might have become attached to RNA though attached to protein in the original tissue.

To investigate this, 1 g of freeze-dry tissue was incubated with 40 ml M perchloric acid at 4^{o}C for 24 hours. According to Ogur and Rosen (1950) this removes RNA, and partially fragments it at the same time. The insoluble residue from this step was then reincubated with 1.5 mg of BDH yeast RNA which had previously been allowed to stand in M perchloric acid for four hours at 4^{o}C producing partial hydrolysis. After the second incubation, both fractions were analysed for uranium to see if any further uranium had been extracted by the RNA fragments, and to assess the likelihood of artefact formation.

(b) results

Analysis showed that 23% of total uranium was extracted when RNA was removed from the tissues under the mild conditions described. This is very much less than the percentage of total uranium found in the form of an RNA complex from the original aqueous extraction under reflux, and means that some kind of transfer is almost certainly taking place. More direct evidence comes from the analysis of the reincubation fractions described in the last paragraph.

The solution containing fresh RNA fragments also contained a further 46% of total uranium, showing clearly that RNA and its degradation products can chelate uranium which was previously attached to other compounds. This means that artefacts will almost certainly be formed in the extraction of freeze-dry tissue under vigorous conditions.

2. Uranium - DNA complex

Although the possibility of artefact formation is high, I checked the amount of uranium bound to total nucleic acids.

(a) method

Total nucleic acids were extracted by the method of Ogur and Rosen (1950), and the percentage uranium in that fraction compared with the amount of uranium bound to RNA alone. One gram of freeze-dry tissue was incubated with 40 ml of 0.5 M perchloric acid at 70°C for 40 min. and the residue and filtrate analysed separately.

(b) results

46% of total uranium extracted in the fraction containing all the nucleic acids. This must be compared with 23% in the RNA fraction.

The significance of this is doubtful, but it may be (taking one extreme conclusion) that some of the uranium is attached to DNA as well as RNA and that the extraction conditions in some way disturb this so that the uranium transfers to RNA. Since the binding sites for uranium on each molecule are substantially the same, a more likely conclusion is that the extraction of DNA acts in the same way as incubating extra RNA fragments with tissue from which RNA has already been extracted.

F. Investigation of subcellular localisation.

Nucleic acids are normally found in specific places within the cell. DNA is found in the nucleus, mitochondria, and chloroplast, RNA is found in the cytoplasm, the microsomes, and to some extent in the nucleus. With the exception of the cytoplasm, these areas are separated from each other by various membranes, and if these are ruptured the various molecules then come in contact.

Freeze-drying itself is a process which causes extensive rupturing. Extraction processes involving reflux for extended periods, will continue this degradation of cellular integrity. Using special techniques, and taking precautions, however, the individual subcellular particles can be removed from fresh tissue, and sorted according to density, by the use of differential centrifugation. The chief difficulty is the variability in the densities of various subcellular organelles from one tissue to another. Fractionation schemes have been evolved which separate the components of a given tissue very satisfactorily, but may fail if applied to a different tissue.

In the evolution of such a method, a great deal of work is necessary. A large number of centrifugations must be carried out, possibly using different suspending media, and buffer strengths; possibly even using a density gradient technique. Every fraction obtained should be monitored by microscopy, and in the case of the very small particles, electron microscopy may be necessary. In spite of this, various standard conditions are in use, which, although not necessarily giving a sharp separation of different particles will, in the majority of cases give fractions containing a majority of the particle sought. In the present work it was not worth while attempting to evolve a detailed fractionation procedure, since only one result was sought,

so the standard conditions were followed, and the results interpreted in this light.

1. Methods

Twelve young Coprosma australis plants were collected from an area in the Buller Gorge not possessing significant mineralisation, and potted using Manawatu loam. After a stabilisation period of three months, a solution of uranyl acetate was applied to the soil at the rate of 150 mg per plant over a period of two weeks, until small purple-brown patches, indicating abnormalities, began to appear on the leaves. The leaves (21g) were removed from the plants, washed in tap water and distilled water, and blended in a buffer at 4°C for 1.5 min., using a Waring blendor. The buffer was tris-HCl, pH 7.3: 0.05 M with respect to tris, and 0.5 M with respect to sucrose. The purpose of the sucrose was to prevent rupture of cells by osmotic shock.

The resulting suspension was strained through several layers of nylon mesh, and centrifuged for 10 min. at 100 g. The residue from this step was labelled 'cell walls'. The supernatant was centrifuged for 10 min. at 1000 g and the precipitated material resuspended in half the original volume of solution, recentrifuged, and the resultant precipitate set aside as 'chloroplasts'. Twenty minutes centrifugation at 20,000 g gave 'mitochondria and nuclei', and 2.5 hours at 80,000 g gave 'microsomes' and a clear solution labelled 'supernatant'. The procedure is that given by Stern (1968), and a flow diagram is given in fig. VI.5. (All steps were at 0-4°C). Each fraction was assayed for uranium.

2. Results

The results are given in table VI.4.

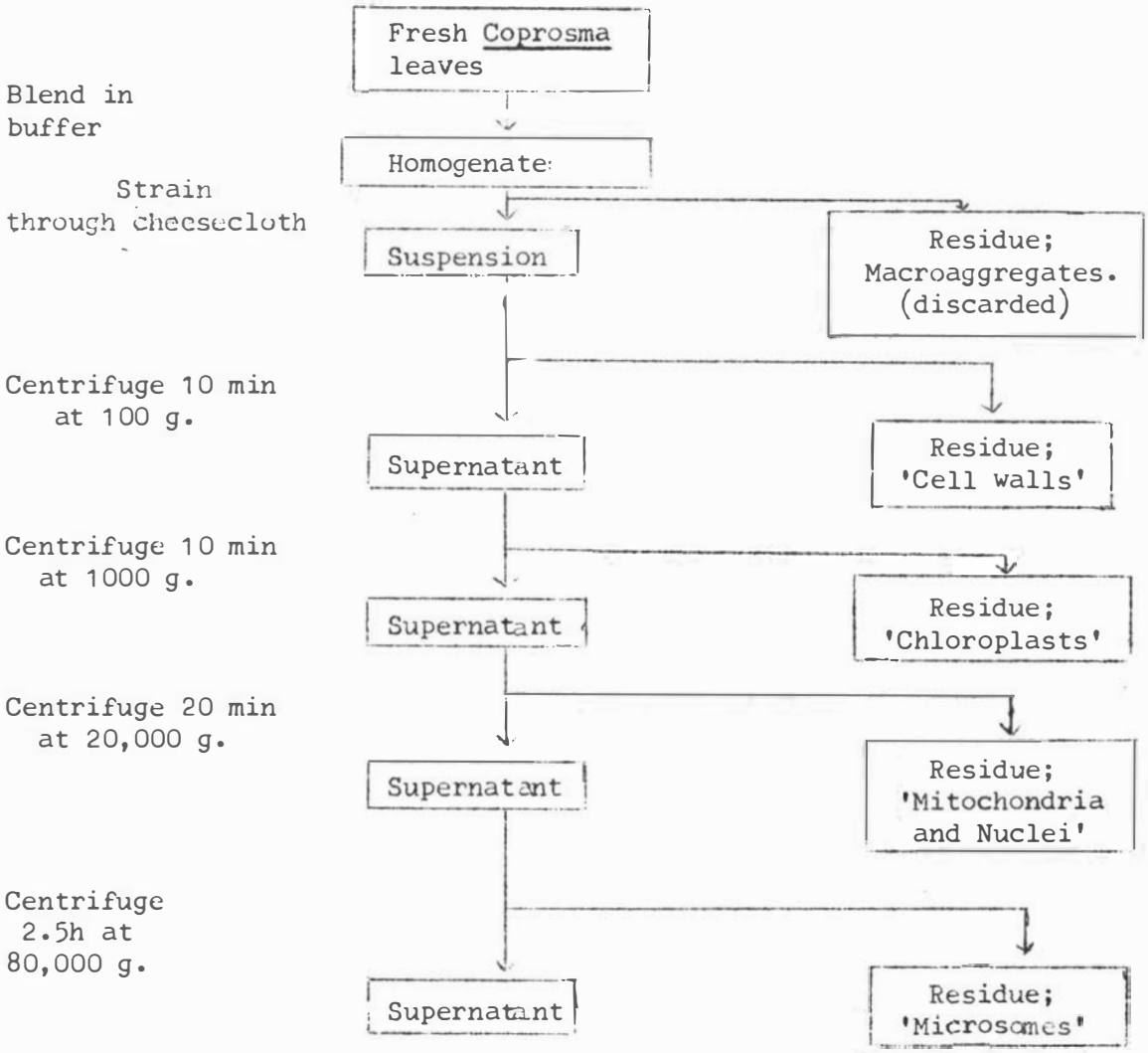


Figure VI.5. Flow diagram for differential centrifugation procedure.

Table VI.4.

Distribution of uranium in various differential centrifugation fractions of C.australis leaves.

Cell fraction	Uranium distribution
"Cell wall fraction"	43
"Chloroplast fraction"	26
"Mitochondria & nuclei fraction"	23
"Ribosome fraction"	0
"Supernatant"	8

Forty-three percent of the uranium was localised in the cell wall, and since there is the possibility of this fraction contaminating later ones, the actual figure may be much higher. The other striking finding is the absence of uranium in the microsomal fraction which would normally contain the bulk of the RNA.

G. Discussion.

This work has shown the probable chemical nature of all but a small fraction of the uranium present in the Coprosma.

The amount unidentified was the 6% collected in the ethanolic fraction of the extraction system, which nearly equals the 8% found in the supernatant of the differential centrifugation system. This is probably some low molecular weight form of uranium, which cannot be further identified from the present evidence. It could be a bicarbonate complex, or organic acid complex, but I think it is very unlikely to be a free aquo-complex.

The aqueous extract of freeze-dry Coprosma contains an RNA complex of uranium.

Uranium in the insoluble residue seems to be bound solely to protein, and since the binding capacity is decreased in acidification, it is concluded that the binding, as suggested in the literature, is at least partially through the carboxyl groups. The other compounds in the residue, apart from protein, do not possess significant binding ability.

Without a very great deal of basic work on the properties of model systems containing various types of proteins, uranium and nucleic acids, it is not possible to estimate with any accuracy the extent uranium can transfer from one of these molecules to another, but such transfer has been qualitatively demonstrated in part E of this section.

If uranium transfers from protein to nucleic acids very readily, then it is possible that in the plant itself all the uranium is bound to

protein of one kind or another, and that nucleic acid complexes are formed only as a result of cell disruption. The other extreme possibility is represented by the figures themselves, which (allowing for the 6% found in the ethanolic fraction) suggest a maximum of 43% is bound to nucleic acids, and a minimum of 51% is bound to protein.

The evidence from the differential centrifugation experiment is that the uranium is mainly attached to the cell walls. None is found in the microsomal fraction, which again suggests that the RNA complex of uranium is an artefact, but without detailed microscopical examination no further insight can be gained. The finding that uranium attaches to the cell walls is very similar to earlier findings for yeast, and suggests that this phenomenon is very widespread indeed, since it has now been observed in higher plants, lower plants, and the animal kingdom (where one of its main toxic effects is mediated through its action on the membranes in the kidney, controlling filtration).

These findings do not offer much in the way of clues that could help in the selection of plants that might selectively accumulate uranium. It can only be postulated that there is a possibility of plants with a high protein content, and perhaps high nucleic acid content being accumulators. This finding will be modified by other factors such as the rate and type of absorption at root level.

This section must hence be considered a contribution to biochemistry rather than biogeochemistry, but the principles used here will need to be extended and further applied in the future. Much further research is required into plant physiology, and the ways inorganic ions exist in cells. Knowledge of these facts will enable a much more rational and successful application of biogeochemical techniques.

GENERAL CONCLUSION

In view of the specialised nature of the various sections of this thesis, the present discussion will not attempt to examine further those results already discussed, but will serve mainly to summarise and conclude.

The general aim was to investigate various aspects of the interaction of a uranium deposit with its environment, and I suggest that this has been largely fulfilled. Of necessity the sections have only covered a small number of the many possible facets of this subject, since an even broader scope would have led to increasing superficiality.

The work is also characterised by the fundamental nature of the investigations. Such topics as the chemical form of uranium in the plant may seem to have little immediate application, but are important if our knowledge of the mechanisms of trace element absorption is to expand. This knowledge will lead to more efficient biogeochemical prospecting techniques, and also a better understanding of plant nutrition in general.

Other findings in the course of this project mainly concern methods of approach to problems. Although the damp and rugged conditions encountered in the Buller Gorge may not be duplicated in many other regions, and the vegetation may be unique, the general lessons may be of use elsewhere. One is that computer techniques enable the rapid discovery of correlations to be made, but that interpretation of these should take into account the way the data are distributed. Another is that the examination of trace element contents may in some cases give valuable though indirect indication of the amount of others, and this could be useful in future biogeochemical prospecting. The section on gamma-ray spectrometry illustrates the potential of any system that gives information about a large number of components simultaneously, but

also shows the care which is necessary, when there is the possibility of contributions from two independent components overlapping. Finally the section on bryophytes shows the possibilities of investigating parts of the 'prospecting prism' other than the accepted ones.

The specific findings and achievements of this project were;

(i) The development of a method of uranium analysis with a sensitivity down in the tens-of-nanograms range.

(ii) The demonstration that four species, C.australis, N.fusca, Q.acutifolia, W.racemosa, appear useful in biogeochemical prospecting; a fifth species, C.serratus being of no use. Beta counts were not a useful measure of the amount of uranium present.

(iii) The demonstration that iron in plant leaves, appears to bear an inverse relationship to the uranium content of both leaves and soil, affording an example of an indirect pathfinder, which may be of general application.

(iv) The development of a new method of prospecting, which uses either peat, or aquatic bryophytes.

(v) The development of a versatile computer programme for correlation calculations and examination of the form of data distributions.

(vi) The development of a method of estimation of gamma-emitting radionuclides which demonstrated for the first time, the presence

of certain isotopes in plants, and showed that most alpha emission is due to uranium.

(vii) The demonstration that uranium in C.australis is chelated by protein and nucleic acids, thus diminishing its toxic effects. Most appears to be attached to the proteins of the cell wall, but on homogenisation, transfer to other compounds may occur.

The value of this thesis should be assessed in relation to its overall contribution to knowledge rather than the contribution of any one section, since although a number of specific conclusions were arrived at, the aim of the work was more general. The ultimate judgement however, must be from those who will in future apply the findings and techniques outlined here, and the author will be satisfied if he has been able to make some small contribution to future mineral exploration.

INDEX OF AUTHORS

SUBJECT INDEX

REFERENCES

Acqua, C. (1913), Atti Accad. Lincei 22 390-392, Nuclear degeneration produced by uranium in plant cells.

Ahrens, L.H. (1954), Geochim. cosmochim. Acta. 5 49-73, The lognormal distribution of the elements.

Ahrland, S. (1951a), Acta chem. scand. 5 199-219, Complex chemistry of the uranyl ion. IV. Complexity of uranyl acetate.

Ahrland, S. (1951b), Acta chem. scand. 5 1151-1167, Complex chemistry of the uranyl ion. V. Complexity of uranyl sulphate.

Anderson, R.Y., Kurtz, E.B. Jr. (1955), Econ. Geol. 50 227-232, Biogeochemical reconnaissance of the Annie Laurie uranium prospect, Santa Cruz County, Arizona.

Anderson, R.Y., Kurtz, E.B. Jr., (1956), Econ. Geol. 51 64-68, A method for determination of alpha radioactivity in plants as a tool for uranium prospecting.

Anon. (1966) Radioactivity Survey Data in Japan. Natl. Inst. radiological Sciences, Chiba (Japan) 13.

Armands, G. (1961), Aktiebolaget Atomenergie, Stockholm. AE36, Geochemical prospecting of a uraniferous bog deposit at Masugnsbyn, northern Sweden.

Armands, G., Landergren, S. (1960), Rep. int. geol. Congr., 21st, Copenhagen, 15 51-66, Geochemical prospecting for uranium in northern Sweden. The enrichment of uranium in peat.

Balabanov. V.I., Kovalevskii, A.L. (1963), Atomn. Energ. 15 432-434, Aerial prospecting of uranium ores in wooded regions.

Baranov, V.I., Kunasheva, K.G., (1954), Trudy biogeokhim. Lab. 10 94-97, Content of radioactive elements of the thorium series in plants.

Beasley, T.M., Palmer, H.E. (1966) Science 152 1062-1063, Lead-210 and Polonium-210 in biological samples from Alaska.

Beck, A.C., Reed, J.J., Willett, R.W. (1958), N.Z.Jl. Geol. Geophys. 1 432-450, Uranium mineralization in the Hawks Crag Breccia of the Lower Buller Gorge region, South Island, New Zealand.

Beer, M., Zobel, C.R. (1961) J.molec. Biol. 3 717-726, Electron Stains. II. Electron microscopic studies on the visibility of stained deoxyribonucleic acid (DNA) molecules.

Beninson, D., Elst, E. Concio, D. (1964), Arg. Rep. Comm. nucl. Energia. Atom Informe. 138 14p, Incorporation of fission products in aquatic plants.

Botova, M.M., Malyuga, D.P., Moiseenko, U.I. (1963), Geokhimiya 4 361-369, Experimental use of the biogeochemical method in prospecting for uranium under desert conditions.

Bovard, P., Grauby, A. (1967), Proc. int. Symp. radioecol. concn. Processes, Stockholm, 1966, Pergamon Press, Oxford. 533-537, The fixation of radionuclides from atmospheric fallout in peat bog sphagnum, Polytrichum and Myriophyllum.

Bowen, H.J.M., Cawse. P.A., Thick, J. (1962), J. exp. Bot. 13 257-267, The distribution of some inorganic elements in plant tissue extracts.

Bowes, W.A., Bales, W.E., Haselton, G.M. (1957) U.S. atom. Energy Commn. Rep. RME-2063 29 p, Geology of the uraniferous bog deposit at Pettit Ranch, Kern County, California.

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

Brooks, R.R., Whitehead, N.E. (1968), J.scient. Instr. Ser. 2 (1) 879-881, A fluorimetric attachment for an atomic-absorption spectrophotometer.

Brunovskii, B.K., Kunasheva, K.G. (1935), Trudy biogeochim. Lab. 3 31-41, Radium content of plants.

Burkholder, P.R. (1963), Nature, Lond. 198 601-613, Radioactivity in some aquatic plants.

Cannon, H.L. (1952), Am. J. Sci. 250 735-770, The effect of uranium-vanadium deposits on the vegetation of the Colorado Plateau.

Cannon, H.L. (1953), Circ. U.S. geol. Surv. 264 1-8, Geobotanical reconnaissance near Grants, New Mexico.

Cannon, H.L. (1955), Bull. U.S. geol. Surv. 1000-D 119-185, Geochemical relations of zinc-bearing peat to the Lockport dolomite, Orleans County, New York.

Cannon, H.L. (1957), Bull. U.S. geol. Surv. 1030-M 399-516, Description of indicator plants and methods of botanical prospecting for uranium deposits on the Colorado Plateau.

Cannon, H.L. (1959), Proc. geochem. expl. Symp., int. geol. Congr., 20th, Mexico City, 235-241, Advances in botanical methods of prospecting for uranium in the Western United States.

Cannon, H.L. (1960a), Bull. U.S. geol. Surv. 1085-A 1-50, The development of botanical methods of prospecting for uranium on the Colorado Plateau.

Cannon, H.L. (1960b), Science 132 5191-5198, Botanical prospecting for ore deposits.

Cannon, H.L. (1964), Bull. U.S. geol. Surv. 1176 127p, Geochemistry of rocks and related soils and vegetation in the Yellow Cat area, Grand County, Utah.

Cannon, H.L., Kleinhampl, F.J. (1956), Prof. Pap. U.S. geol. Surv. 300 681-686, Botanical methods of prospecting for uranium.

Cannon, H.L., Starrett, W.H. (1956), Bull. U.S. geol. Surv. 1009-M 391-407, Botanical prospecting for uranium on La Ventura Mesa, Sandoval County, New Mexico.

Cassie, R.M. (1954), Austr. J. Mar. Freshwater Res. 5 513-522, Some uses of probability paper in the analysis of size frequency distributions.

Castella-Bertran, E., Rife-Bertran, M. (1950), An. Fac. Vet. Univ. Madr. 2 64-69, Actions of some metals and metalloids on acid phosphomonoesterase of dog liver.

Ceriotti, G. (1952), J. biol. Chem. 198 297-303, A microchemical determination of desoxyribonucleic acid.

Chamberlain, J.R. (1964), Bull. Can. Dep. Mines Technol. 118 19p, Hydrogeochemistry of uranium in the Bancroft-Haliburton region Ontario.

Chandler, R.P., Wieder, S. (1963), Radiol. hlth. Data 4 317-324, Radionucleides in the Northwestern Alaska food chain, 1959-61.

Chapman, R.M., Shacklette, H.T. (1960), Prof. Pap. U.S. geol. Surv. 400-B 104-107, Geochemical exploration in Alaska.

Chirikov, F.V. (1913), Izv. Result. Veget. Opytov. Lab. Rabot. 9 431-435, Stimulants of plant growth.

Cleary, J.J., Hamilton, E. (1968), Analyst 93 235-236, Loss of polonium-210 on dry ashing rat tissues in a muffle furnace.

Cohen, N.E., Brooks, R.R., Reeves, R.D. (1967), N.Z. Jl. Geol. Geophys. 10 732-741, The occurrence of beryllium in the Hawks Crag Breccia of the Lower Buller Gorge region of New Zealand.

Cohen, N.E., Brooks, R.R., Reeves, R.D. (1969), Econ. Geol. 64 519-525, Pathfinders in geochemical prospecting for uranium in New Zealand.

Cooper, J.A., Wagman, N.A., Palmer, H.E., Perkins, R.W. (1968), Hlth. Phys. 15 419-433, The application of solid-state detectors to environmental and biological problems.

Coote, G.E. (1967), Rep. N.Z. Inst. nucl. Sciences INS-R-42 51p, Ge(Li) gamma ray detectors; their fabrication, commissioning, and maintenance.

Coote, G.E. Cohen, N.E., Whitehead, N.E. (1970) N.Z.Jl. Geol. Geophys. (in press), Investigation of uranium ores by high resolution gamma-ray spectrometry.

Dean, M.H. (1966), J.Ecol. 54 589-595, A survey of the uranium content of vegetation in Great Britain.

Debnam, A.H. (1955), Rec. Commonw. Austr. Dep. natn. Dev., Bur. miner. Resour. Geol. Geophys. 1955/43 24p, Biogeochemical prospecting investigations in the northern territory 1954.

DeKock, P.C. (1964), Outlook Agr. 4 93-98, The physiological significance of the potassium-calcium relationship in plant growth.

Dezsi, Z. (1963), Fiz. Szle. 13 298-304, The determination of the radioactive materials content of natural waters.

Drobkov, A.A. (1939), Trav. Lab. biogeochim. Acad. Sci. 5 201-204, Effect of Uranium, radium and actinium on the yield of peas.

Drobkov, A.A. (1949), Sov. Agron. 7 75-79, The role of natural radioactive elements in the life of plants.

Duvigneaud, P. (1959), Bull. Soc. r. Bot. Belg. 91 111-134, Plant cobaltophytes in Upper Katanga.

Eckel, E.B., Williams, J.S., Galbraith, F.W., et al. (1949), Prof. Pap. U.S. geol. Surv. 219 179p. Geology and ore deposits of La Plata District, Colorado.

Ewan, G.T., Tavendale, A.J. (1964), Can. J. Phys. 42 2286-2331, High-resolution studies of gamma-ray spectra using lithium-drift germanium gamma-ray spectrometers.

Feigl, F. (1967), Spot tests in inorganic analysis. 5th ed., Elsevier Publ. Co, Amsterdam, 600p.

Feldman, T., Koval, L. (1962), Rep. U.S. atom. Energy. Commn. UR-611 15 p, Reaction of the uranyl ion with amino acids - bidentate carboxylate chelation.

Fielding, H.C. (1961), British patent 865331 Ap. 12 1961, Coordinating resins for extracting cations from solutions.

Fisher, R.A. (1941), Statistical methods for research workers, 8th ed., Oliver and Boyd, Lond. 239p.

Fisher, R.A., Yates, F. (1953), Statistical tables for Biological, Agricultural, and Medical Research, 4th ed. Oliver and Boyd, Lond. 98p.

Fix, P.F. (1955), Proc. int. Conf. peaceful Uses atom. Energy, Geneva 1955 6 788-791, Geochemical prospecting for uranium by sampling ground and surface masses.

Fletcher, M.H. (1954), Bull. U.S. geol, Surv. 1006 51-68, A study of critical factors in the direct fluorimetric determination of uranium.

Fletcher , M.H., May, I., Slavin, M. (1954), Bull. U.S. geol. Surv. 1006 85-92 A transmission fluorimeter for use in the fluorimetric method of analysis for uranium.

Fortescue, J.A.C., Hornbrook, F.H.W. (1967), Geol. Surv. Pap. Can. 67-23 pt I 143p, Progress report on biogeochemical research at the Geological Survey of Canada 1963-1966.

Francis, C.W., Chesters, G. (1967), J. Agr. food. Chem. 15 704-706, Radioactive ingrowth of polonium-210 in tobacco plants.

Francis, C.W., Chesters, G., Erhardt, W.H. (1968), Environ. Sci. Technol. 2 692-697, Polonium-210 entry into plants.

Froelich, A.J., Kleinhampl, F.J. (1960), Bull. U.S. geol. Surv. 1085-B 51-84, Botanical prospecting for uranium in the Deer Flat area, White Canyon District, San Juan County, Utah.

Gabay, J.J., Dapolito, J.A., Sax, N.I. (1965), Annual Bioassay Meeting, Albequerque, New Mexico, Oct. 7-8, 1965.

Gabay, J.J., Sax, N.I. (1969), Hlth. Phys. 16 812-813, Retention of radium, due to ingestion of Brazil nuts.

Gaddum, J.H. (1945), Nature Lond. 156 463-466, Lognormal distributions.

Garder, K. Skulberg, O. (1966), Acta hydrobiol. Krakow 62 50-69, Experimental investigation on the accumulation of radioisotopes by fresh-water biota.

Geiger, E.L. (1959), Hlth. Phys. 1 405-408, Radioassay of uranium and plutonium in vegetation, soil and water.

Gest, H., Glendenin, L.E. (1951), Natl. nucl. Energy. Ser. Div IV, 9 Radiochemical studies on the Fission Products, Book I, 170-175, Coseparation of aqueous fission products with manganese dioxide.

Glover, N. (1953), Natl. nucl. Energy Ser. Div. VI, Vol. I, Pharmacology and toxicology of uranium compounds, Book 3, 1139-1166, Isolation of microquantities of uranium by precipitation with protein prior to fluorometric determination.

Goldsztein, M. (1957), Bull. Soc. fr. Miner. Cristallogr. 80 318-324, Geobotanical prospecting for uranium at Esterel.

Grimaldi, F.S., May, I., Fletcher, M.H. (1952), Circ. U.S. geol. Surv. 199 20p, U.S. Geological Survey fluorimetric methods of analysis.

Grimaldi, F.S., May, I., Fletcher, M.H., Titcomb, J. (1954), Bull. U.S. geol. Surv. 1006 1-9, Summary of methods of analysis for the determination of uranium and thorium.

Grodzinskii, D.M. (1959), Ukr. biokhim. Zh. 16 30-81, Natural radioactivity of mosses and lichens.

Grodzinskii, D.M., Golubkova, M.G. (1963), Mikro-element. sel'sk.-khoz. Med. Sb. 461-464, Determination of uranium in soils and plants.

Grodzinskii, D.M., Golubkova, M.G. (1964), Fiziol.-biokhim. Osobennosti Deistviya. yadern. Izluchen. Rast. Akad. Nauk. Ukr. SSR Inst. Fiziol. Rast. 135-146, The nature of existing radioactivity of the soil and plants in the USSR.

Guest, R.J., Zimmerman, J.B. (1955), Analyt. Chem. 27 931-936, Determination of uranium in uranium concentrates - use of ethyl acetate.

Gulyakin, I.V., Korovkina, A.V. (1963), Dokl. Mosk. sel'.-khoz. Akad. 84 327-331, The absorption of polonium by oats.

Gulyakin, I.V., Dergunov, I.D., Moroz, V.D. (1968), Izv. timiryazev. sel'.-khoz. Akad. 84-93, Behaviour of radioactive fission products in some soils of the sierozem zone of Uzbekistan.

Guzman-Barron, E.S. (1951), Natl. nucl. Energy Ser. Div. IV. 23 Toxicology of uranium 40-44, Mechanism of action of uranium and the transport of uranium to the tissues.

Gvozdanovic, S.M., Overton, T.R., Spiers, F.W. (1967), Rep. U.K. atom. Energy Auth. AERE-R5474 175-189, A study of the concentrations of ^{226}Ra and ^{228}Th in the living environment.

Hastings, C. (1955), Approximations for digital computers. Princeton University Press, Princeton, N.J. 201 p.

Herdan, G. (1949), Quality control by statistical methods. Nelson and sons, Lond. 251p.

Hevesy, G. (1923), Biochem. J. 17 439-445, The absorption and translocation of lead by plants. A contribution to the application of the method of radioactive indicators in the investigation of the change of substance in plants.

Hill, C.R. (1960), Nature, Lond. 187 211-212, Lead-210 and polonium-210 in grass.

Hill, C.R. (1962), Hlth. Phys. 8 17-25, Identification of α-emitters in normal biological materials.

Hoffmann, J. (1941), Naturwissenschaften 29 403-404, The fixing of uranium by living fresh water algae.

Hojo, N., Kido, K., Takizawa, N. (1958), Shinshu Igaku Zasshi 8 133-135, Selective resins containing oxygen atoms as electron donor for uranium.

Horvath, E. (1960), Atomki. Kozl. 2 177-183, Uranium adsorption on peat in natural waters containing uranium traces.

Hurwitz, L., Rothstein, A. (1951), J. cell. comp. Physiol. 38 437-501, The relationship of the cell surface to metabolism, VII. The kinetics and temperature characteristics of uranium inhibition.

Huxley, H.E., Zubay, G. (1960), J. molec. Biol. 2 10-18, Electron microscope observations on the structure of microsomal particles from Escherichia coli.

Ishimori, T, Watanabe, K., Nakamura, E. (1960), Bull. chem. Soc. Jap. 33 636-644, Inorganic extraction studies on the system between tri-n-butyl phosphate and hydrochloric acid.

Jacobson, L., Overstreet, R. (1948), Soil. Sci. 65 129-134, The uptake by plants of plutonium and some products of nuclear fission adsorbed on soil colloids.

Jurain, G. (1962a), Commn. Energie atom. Rap. 2154 349p, The U-Ra and Th families in the southern Vosges - application of some results in the prospecting for U deposits.

Jurain, G. (1962b), D.Sc. thesis, University of Nancy, France. Contribution to the geochemical knowledge of the uranium-radium, and thorium families in the meridional Vosges. The application of certain results in prospecting for uranium deposits.

Kauranen, P., Miettinen, J.K. (1967), Proc. int. Symp. radioecol. concn. Processes, Stockholm, 1966, Pergamon Press, Oxford. 275-280, ^{210}Po and ^{210}Pb in environmental samples in Finland.

Keaton, C.M. (1937), Soil Sci. 43 401-411, Influence of lead compounds on the growth of barley.

Kendall, M.G., Stuart, A. (1963), The advanced theory of statistics, Vol. I, 3rd ed, Griffin, Lond. 439p.

Kleinhampl, F.J. (1962), Bull. U.S. geol. Surv. 1085-D 105-188, Botanical prospecting for uranium on South Elk ridge, San Juan County, Utah.

Kleinhampl, F.J., Koteff, C. (1960), Bull. U.S. geol. Surv. 1085-C 85-104, Botanical prospecting for uranium in the Circle Cliffs area, Garfield County, Utah.

Koczy, G. (1950), Ost. Akad. Wiss. math. Naturw. 158 113-121, Further uranium determinations in samples of sea water.

Konstantinov, V.M. (1963), Sov. Geol. 6 151-155, Feasibility of using a biogeochemical survey in prospecting for uranium in arid territories.

Korkisch, J., Arrhenius, G. (1964), Analyt. Chem. 36 850-854, Separation of uranium, thorium, and the rare earths, by anion exchange.

Kovalevskii, A.L. (1962), Izv. Sib. Otd. Akad, Nauk. SSSR. 108-114, Naturally radioactive elements in plants.

Kovalevskii, A.L. (1964), Trudy Konf. Pochv. Sib. dal'n. Vost., Novosib. 53-60, Geochemistry of radioactive elements in soils.

Kovalevskii, A.L. (1965), Izv. sib. Otd. Akad. Nauk. SSSR biol.-med. Nauk 1 43-47, Absorption of radium by various kinds of plants.

Koval'skii, V.V., Vorotnitskaya, I.E. (1965), Geokhimiya 6 724-732, Biogenic migration of uranium in the lake Issyk-Kul.

Koval'skii, V.V., Vorotnitskaya, I.E. (1966), Ukr. biokhim. Zh. 38 419-424, The biogenic migration of uranium.

Koval'skii, V.V., Vorotnitskaya, I.E., Lekarev, V.S. (1967), Proc. int. Symp. radioecol. concn. Processes, Stockholm 1966. Pergamon Press, Oxford. 329-332, Biogeochemical food chains of uranium in aquatic and terraneous organisms.

Kumar Dutt, N., Goswami, N. (1959), Z. anorg. allg. Chem. 298 265-269, The salicylato complexes of uranium (VI).

Kunasheva, K.G. (1939), Trav. Lab. biogeochim. Acad. Sci. URSS 5 187-196, A new method for measuring radioactivity of plant and animal ash by α rays.

Kunasheva, K.G. (1944), Trav. Lab. biogeochim. Acad. Sci. URSS 7 98-105, Radium content in plant and animal organisms.

Lardy, H.A., Anderson, T.F. (1942), Science 95 330-331, Effect of coloured ions on the photoinactivation of invertase.

Lecoq, J.J., Bigotte, G., Hinault, J., Leconte, J.R. (1958) Proc. int. Conf. peaceful Uses atom. Energy, 2nd, Geneva, 1958 2 744-786, Prospecting for uranium and thorium minerals in desert countries, and in the equatorial forest regions of the French Union.

Lederer, C.M., Hollander, J.M., Perlman, I. (1969), Table of Isotopes, 6th ed. Wiley, New York, 594p.

LeRoy, L.W., Koksoy, M. (1962), Econ. Geol. 57 107-113, The Lichen as a possible plant medium for mineral exploration.

Leutwein, F., Weise, L. (1962), Geochim. cosmochim Acta 26 1333-1348, Hydrogeochemical investigations of mine and surface waters of the Erzgebirge.

Li, N.C., Westfall, W.M. Lindenbaum, A., White, J.M., Schubert, J. (1957), J. Am. chem. Soc. 79 5864-5870, Manganese-54, uranium-233 and cobalt-60 complexes of some organic acids.

Lisitsin, A.K., Kruglov, A.I., Panteleev, V.M., Sidel'nikova, V.D. (1967), Lithol. mineral Res. 3 360-370, Conditions of uranium accumulation in lowmoor oxbow peat bogs.

Loew, O. (1904), Bull. Coll. Agr. Tokyo 6 161-175, On the treatment of crops by stimulating compounds.

Lounamaa, J. (1956), Annls. bot. Soc. zool.-bot. fennicae Vanamo 29 196p, Trace elements in plants growing wild on different rocks in Finland, a semiquantitative spectrographic survey.

Lowman, F.G. (1960), Disposal radioact. Wastes 2 105-138, Marine biological investigations at the Eniwetok Test site.

Lyon, G.L. (1969), Unpublished Ph.D. Thesis, Massey University. Trace elements in New Zealand plants.

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

Lyon, G.L., Brooks, R.R., Peterson, P.J., Butler, G.W. (1968), Pl. Soil 29 225-240, Trace elements in a New Zealand serpentine flora.

Makarov, M.S. (1965), Vop. rud. Geofiz., Minist. Geol. Okhrany Nedr. SSSR 33-39, Biogeochemical prospecting for uranium deposits in an area of the USSR.

Malyuga, D.P. (1964), Biogeochemical methods of prospecting. Consultants Bureau New York. 205p.

Mamulea, O., Buracu, O. (1967), Dari Seama sedintel. Repub. Soc. Rom. Commn. geol. 52 237-244, The experimental application of the biogeochemical method in prospecting for uranium deposits.

Mann, H.A., Janarek, F.J., Helenberg, H.W. (1966), IEEE Trans. nucl. Sci. 13 336-350, Preparation of large volume planar germanium detectors and results of their use in nuclear physics experiments.

Manskaya, S.M., Drozdova, T.V., Emelianova, M.P. (1956), Geokhimiya 4 10-24, Binding of uranium by humic acids and by melanoidines.

Marquez, L., Costa, N.L., Almeida, I.G. (1959), Nuovo cim. 11 111-112, Presence of Cobalt-57 in the atmosphere.

Marsden, E. (1964), The natural radiation environment. W.M.Rice University, 807-824, Radioactivity of some rocks, soils, plants and bones.

Mathevon, G., Rothe, J.P., Siffert, P., Coche, A. (1967), Compt. rend. Acad. Sci. (Paris) 264 1753-1756, Determination of the radioactivity of rocks by gamma spectrometry with the help of a germanium (lithium compensated) detector.

Medvedeva, E., Starobina, T.M., Gromov, B.V. (1964), Trudy Mosk. Khim.-tekhnol. Inst. 47 145-150, Study of the extraction of U^{6+} by solutions of tri-n-octylamine in the presence of Fe^{2+} and Mn^{2+}.

Meehan, W.R., Smythe, L.E. (1967), Environment. Sci. Technol. 1 839-844, Occurrence of beryllium as a trace element in environmental materials.

Menzel, R.G. (1965), Hlth. Phys. 11 1325-1332, Soil-plant relationships of radioactive elements.

Mikawa, Y. (1924), Biochem. Z. 149 540-549, The action of uranium acetate. II. Action on certain enzymes.

Mistry, K.B., Gopal-Ayengar, A.R., Bharathan, K.G. (1965), Hlth. Phys. 11 1459-1470, On the radioactivity of plants from the high radiation areas of the Kerala coast and adjoining regions. II. Studies on the uptake of α and β emitters.

Miyake, Y., Sugimura, Y., Tsubota, H. (1964), The Natural radiation environment. W.W. Rice University 219-225, The content of uranium radium and thorium in river waters in Japan.

Moiseenko, U.I. (1959), Geochemistry 1 117-121, Experimental application of the biogeochemical survey to the search for uranium in swampy areas.

Moore, F.L. (1958), Analyt. Chem. 30 908-911, Liquid-liquid extraction of uranium and plutonium from hydrochloric acid solution with tri (iso-octyl) amine. Separation from thorium and fission products.

Morgan, P.G., Bartrum, J.A. (1915), Bull. N.Z. geol. Surv. 17 210p, The Geology and mineral resources of the Buller-Mohikinui subdivision, Westport division.

Muntz, J.A., Guzman-Barron, E.S. (1951), Natl. nucl. Energy Ser. Div. IV. 23, The toxicology of uranium 182-198, Transport of uranium to the tissues.

Muntz, J.A., Singer, T.P., Guzman-Barron, E.S. (1947), Natl. nucl. Energy Ser. Div. IV. 23 246-261, Effect of uranium on the metabolism of yeast and bacteria.

Nemodruk, A.A., Vorotnitskaya, I.E. (1962), Zh. analit. Khim. 17 481-485, An extraction luminescent method for determining uranium in soils, silts, plants, and animal tissues.

Neuman, W.F., Fleming, R.W., Carlson, A.B., Glover, N. (1948), J. biol. Chem. 173 41-52, The fluorophotometric determination of uranium in biological material.

Nicolas, D., Brooks, R.R. (1969), Proc. Australas. Inst. Min. Metall. 231 59-66, Biogeochemical prospecting for zinc and lead in the Te Aroha region of New Zealand.

Nichols, E.L., Slattery, M.K. (1926), J. opt. Soc. Am. 12 449-466, Uranium as an activator.

Ogur, M., Rosen, G. (1950), Arch. Biochem. Biophys. 25 262-276, The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid.

Ohashi, S. (1961), Min. Inst. Jap. Jl. 77 862-867, The fluorometric method in chemical prospecting for uranium deposits. III. Chemical treatments.

Ohashi, S., Murakami, Y. (1960), Genshiryoku Kogyo. 6 41-47, Progress in geochemical prospecting methods.

Ono, T., Kawai, H., Kawai, M. (1959), Acta Sch. med. Gifu 9 41-54, Inorganic constituents in plants. I. Qualitative determination by spectrographic analysis and quantitative determination by polarography.

Ostle, D. (1954), Min. Mag. Lond. 91 201-208, Geochemical prospecting for uranium.

Parker, R.P. (1962), Phys, Med. Biol. 7 301-311, A method of low energy γ-ray spectrometry applicable to the measurement of biological samples.

Peacock, J.D. (1961), Nature, Lond. 191 1189-1190, Uranium in British surface and underground waters.

Pearce, S.C. (1945), Nature, Lond. 156 747, Lognormal distributions.

Penna-Franca, E. (1959), Proc. int. Am. Symp. peaceful Appl. nucl. Energy, 2nd 65-75, Analysis of radioactive contaminants in biological materials.

Penna-Franca, E., Almeida, J.C., Becker, J., Emmerich, M., Roser, F.X., Kegel, G., Hainsberger, L., Cullen, T.L., Petrow, H., Drew, R.T., Eisenbud, M. (1965), Hlth. Phys. 11 699-712, Status of investigations in the Brazilian areas of high natural radioactivity.

Penna-Franca, E., Fizman, M., Lobao, N., Costa-Ribeiro, C., Trindade, H., Dos Santos, P.l., Batista, D. (1968), Hlth. Phys. 14 95-100, Radioactivity of Brazil nuts.

Penna-Franca, E., Gomes de Freitas, O. (1963), Nature, Lond. 197 1062-1063, Radioactivity of biological materials from Brazilian areas rich in thorium compounds.

Perkins, R.W. (1961), Hlth. Phys. 7 81-86, A large well crystal for the direct measurement of trace amounts of radioisotopes in environmental samples.

Persson, N. (1948), Revue bryol. lichen. 17 76-78, On the discovery of Merceya ligulata in the Azores, with a discussion of the so-called "Copper mosses".

Phelps, P.L. (1967), Rep. U.S. atom. Energy. Commn. URCL-70544 19p, Gamma ray spectrometers for the assay of complex mixtures of low concentrations of radionuclides in environmental and biological mixtures.

Pijck, J., Hoste, J., Gillis, J. (1960), Proc. int. Symp. Microchem. Birmingham University, 1958 48-58, Trace element losses during mineralisation of organic material - a radiochemical investigation.

Poole, R.J., Poel, L.W. (1965), J. exp. Bot. 16 453-461, Carbon dioxide and pH in relation to salt uptake by beet root tissue.

Potter, G.D., McIntyre, D.R., Pomeroy, D. (1969), Hlth. Phys. 16 297-300, Transport of fallout radionuclides in the grass-to-milk food chain studied with a germanium lithium-drifted detector.

Price, G.R., Ferretti, R.J., Schwartz, S. (1953), Analyt. Chem. 25 322-333, Fluorophotmetric determination of uranium.

Radford, E.P., Hunt, V. (1964), Science 143 247-249, Polonium-210; a volatile radioelement in cigarets.

Raikov, L., Yanachkova, M., Iotov, M., Petkov, T. (1966), Pochv. Agrokhim. 1 155-162, Uranium and radium uptake by some crops from contaminated soil.

Ravera, O. (1966), G. Fis. Sanit. Radioprot. Radiaz. 10 162-165, Macrophytes as indicators of the radioactive contamination of lake waters.

Rickard, W.H., Davis, J.J., Hanson, W.C., Watson, D.G. (1964), Rep. U.S. atom. Energy Commn. Contr. AT (45-1) 1350, Gamma emitting radionuclides in Alaskan tundra vegetation, 1959, 1960, 1961.

Robert, M. (1968), CEA-R 3692 57p, Use of a uranium ore for the overall calibration of gamma spectrometers.

Robertson, D.E. (1968), Analyt. chim. Acta. 42 533-536, The adsorption of trace elements in sea water on various container surfaces.

Roger, H. (1909), Compt. rend. Soc. biol. 65 388-389, The action of uranium acetate upon some amylolytic ferments.

Rogers, J.J.W., Adams, J.A.S. (1963), Geochim. cosmochim. Acta 27 775-783, Lognormality of thorium concentrations in the Conway granite.

Roser, F.X. (1966), Rep. U.S. atom. Energy Commn. NYO-2577-3 101p, A study of natural radioactivity in Brazil.

Roslyakov, V.S., Ezhova, M.P. (1966), Sov. Radiochem. 8 332-336, Concentration of uranium from water samples of large volume directly at the source.

Rothstein, A., Frankel, A., Larrabee, C. (1948), J. cell. comp. Physiol. 32 261-274, The relationship of the cell surface to metabolism. III. Certain characteristics of the uranium complex with cell surface groups of yeast.

Rothstein, A., Larrabee, C. (1948), J. cell. comp. Physiol. 32 247-259, The relationship of the cell surface to metabolism. II. The cell surface of yeast as the site of inhibition of glucose metabolism by uranium.

Rothstein, A., Meier, R. (1951), J. cell. comp. Physiol. 38 245-247, The relationship of the cell surface to metabolism. VI. The chemical nature of uranium-complexing groups of the cell surface.

Salmi, M. (1955), Bull. Commn. Geol. Finland. 169 5-34, Prospecting for bog-covered ore by means of peat investigations.

Salmi, M. (1956), Bull. Commn. Geol. Finland. 175 5-22, Peat and bog plants as indicators of ore minerals in Vihanti ore field in western Finland.

Samoilova, A.P. (1961), Razvedka, Okhrana Nedr. 27 11-15, Calculation of factors in biogeochemical research.

Samsoni, Z. (1967), Mikrochim. Acta 88-97, Investigation of factors affecting the accuracy and sensitivity of the fluorimetric determination of uranium.

Sani, A.R. (1966), Nucleus (Lahore) 3 102-104, Uranium from seawater.

Sato, T. (1965), J. appl. Chem, Lond. 15 489-495, Extraction of uranium and thorium from nitric acid solutions by tri-n-butyl phosphate.

Sato, T. (1966), J. appl. Chem. Lond. 16 53-57, Extraction of uranium and thorium from HCl solutions by tri-butyl phosphate.

Saukov, A.A. (1955), Proc. int. Conf. peaceful Uses atom. Energy, Geneva, 1955, 756-759, Radiohydrological method in prospecting for uranium deposits.

Shacklette, H.T. (1965), Bull. U.S. geol. Surv. 1198-D 21p, The elemental content of bryophytes.

Shacklette, H.T. (1967), Bull, U.S. geol. Surv. 1198-G 17p, Copper mosses as indicators of metal concentrations.

Singer, T.P., Muntz, J.A., Meyer, J., Gasvoda, B., Guzman-Barron E.S. (1947), Natl. nucl. Energy Ser. Div. IV. 23 The toxicology of uranium 208-245, The reversible inhibition of enzymes by uranium.

Skulmowski, J., Wiercinski, J. (1966), Annls. Univ. Mariae Curie-Sklodowska, Sect. DD 21 73-81, Determination of trace elements in feeds by the polarographic method. V. The trace elements cobalt, copper, nickel, zinc, cadmium, and bismuth in silage produced in the Lublin Voivodeship.

Sokolova, A.I., Khramova, V.V. (1961), Trudy Sverdlosk. gorn. Inst. 40 107-115, Biogeochemical studies.

Stary, J. (1960), Colln. Czech. chem. Commun. Engl. Edn. 25 2630-2641, Determination of the composition and of the stability constants of metal complexes by the extraction method.

Stary, J., Balek, V. (1962), Colln. Czech. chem. Commun. Engl. Edn. 27 809-815, Research of α-hydroxy acid uranium (VI) complexes by the extraction method.

Stern, H. (1968), Biochemist's Handbook Spon, Lond. 1192p, Fractionation of plant cells.

Svennerholm, L. (1957), Biochim. biophys Acta 24 604-611, Estimation of sialic acids. II. Colorimetric resorcinol-hydrochloric acid method.

Szalay, A. (1957), Acta phys. Acad. Sci. Hung. 8 25-37, The role of humus in the geochemical enrichment of uranium in coal and other bioliths.

Talipov, Sh. T., Krukovskaya, E.L., Rasuleva, Sh. (1961) Uzbek. khim. Zh. 18-24, Solubility of cerium (III) oxalate in solutions of iron (III), aluminium and uranyl nitrates at 25°C.

Tennant, C.B., White, M.L. (1959), Econ. Geol. 54 1281-1290, Study of the distribution of some geochemical data.

Thamer, B.J. (1956), Rep. U.S. atom. Energy Commn. LA-1996 85 p, Spectrophotmetric and solvent extraction studies of uranyl phosphate complexes.

Tilak, V.V.S.S., Aswathanarayana, U. (1963), Proc. Indian Acad. Sci, Sect. A 57 34-43, Chemical studies in the uranium prospect at Umra, Kajasthan. I Geochemistry of waters.

Timperley, M.H., Brooks, R.R., Peterson, P.J. (1970), Econ. Geol. (in press), Biogeochemical prospecting for copper and nickel in New Zealand.

Tserkovnitskaya, I.A., Bykhovtseva, T.T. (1964), Metod. Kolichestv.-Opred. Elementov. Leningr. Gos. Univ. 101-106, Extractive separation of uranium with high molecular weight amines.

Tso, T.C., Fisenne, I. (1968), Radiat. Bot. 8 457-462, Translocation and distribution of lead-210 and polonium-210 supplied to tobacco plants.

Tso, T.C., Hallden, N.A., Alexander, L.T. (1964), Science 146 1043-1045, Radium-226 and polonium-210 in leaf tobacco and soil.

Tso, T.C., Harley, N., Alexander, L.T. (1966), Science 153 880-882, Source of Pb-210 and Po-210 in tobacco.

Tso, T.C., Steffers, G.L., Ferri, E.S., Baratta, E.J. (1968), Agron. J. 60 647-649, Agronomic factors affecting polonium-210 and lead-210 levels in tobacco. I. Soil and fertilizer.

UKAEA. (1960) British patent 948962 Feb. 5 1964, Appl. Jan 22 1960, Polymers derived from poly (vinylphenols).

Umemoto, S., Mifune, M. (1953), Rep. balneol. Lab. Okayama Univ. 12 11-14, Chemical studies on the thermophilic algae. I. Inorganic constituents.

Valentine, R.C. (1958), J. r. microsc. Soc. 78 26-29, Quantitative electron staining of virus particles.

Vera-Palomino, J., Palomares-Delgado, F., Petrement-Eguiluz, J.C. (1964), Rep. U.S. atom. Energy Commn. JEN-136-DQ/I-40 25p, Solvent extraction systems applied to uranium analysis. I. Extraction studies with tributyl phosphate - methyl isobutyl ketone.

Verkhovskaya, I.N., Vavilov, P.P., Maslov, V.I. (1967), Proc. int. Symp. radioecol. concn. Processes, Stockholm 313-328, The migration of natural radioactive elements under natural conditions, and their distribution according to biotic and abiotic environmental components.

Vernadskii, V.I., Brunovskii, B.K., Kunasheva, K.G. (1937), Nature, Lond. 140 317-318, Concentration of mesothorium 1 by duckweed (Lemna).

Von Neumann, J., Morgenstein, O. (1953), Theory of games and economic behaviour. Princeton University Press, Princeton, N.J. 641p.

Von Wazer, J.R., Campanella, D.A. (1950), J. Am. chem. Soc. 72 655-663, Structure and properties of the condensed phosphates. IV. Complex-ion formation, in polyphosphate solutions.

Walker, R.B. (1954), Ecology 35 259-266, The ecology of serpentine soils. II. Factors affecting plant growth on serpentine soils.

Walker, R.B., Walker, H.M., Ashworth, P.R. (1955), Pl. Physiol. 30 214-221, Calcium-magnesium nutrition with especial references to serpentine soils.

Wallace, G., Coote, G.E. (1969), Nucl. Instrum. Methods 74 353-354, Efficiency calibration of Ge(Li) detectors using a radium source.

Wannemacher, R.W., Banks, W.L., Wunner, W.H. (1965), Analyt. Biochem. 11 320-326, Use of a single tissue extract to determine cellular protein and nucleic acid concentrations and rate of amino acid incorporation.

Warren, H.V., Delavault, R.E. (1949), Bull. geol. Soc. Am. 60 531-560, Further studies in biogeochemistry.

Watters, R.L., Johnson, J.E. (1968), Rep. U.S. atom. Energy Commn. Contr. AT (11-1)-1733 COO-1733-1 27p, A study of unsupported polonium-210 for ion exchange in soil and uptake in vegetation.

Webb, D.A. (1937), Scient. Proc. r. Dubl. Soc. 21 505-539, Studies on the ultimate composition of biological material. Pt.II. Spectrographic analysis of marine invertebrates, with special reference to the chemical composition of their environment.

Webb, D.A., Fearon, W.A. (1937), Scient. Proc. r. Dubl. Soc. 21 487-503, Studies on the ultimate composition of biological material. Pt. I. Aims, scope and methods.

Webber, G.R. (1965), Geochim. cosmochim. Acta 29 229-248, Second report of analytical data for CAAS syenite and sulphide standards.

Wellman, H.W. (1950), N.Z. Jl. Sci. Technol. B 32 11-38, Ohika beds and past Hokonui orogeny.

Whitehead, N.E., Brooks, R.R. (1968), Econ. Geol. 64 50-56, A comparative evaluation of scintillometric, geochemical and biogeochemical methods of prospecting for uranium.

Whitehead, N.E., Brooks, R.R. (1969), Bryologist 72 501-507, Aquatic bryophytes as indicators of uranium mineralisation.

Whittle, A.W.G. (1960), AMDEL Rep. 33 29p, Mineralogy and petrology of the Buller Gorge uranium field.

Wiesner, R. (1938), Sber. Akad. Wiss. Wien. 147 521-528, Determination of radium content of marine algae.

Wilson, D.O., Cline, J.E. (1963), Ann. Rep. Hanford Biol. Research 187. Removal of ^{239}Pu, ^{185}W, ^{210}Pb from soil by plants and ion exchange solutions.

Wodzicki, A. (1959a), N.Z.Jl. Geol. Geophys. 2 385-393, Radioactive boulders in Hawks Crag Breccia.

Wodzicki, A. (1959b), N.A.Jl. Geol. Geophys. 2 602-612, Geochemical prospecting for uranium in Lower Buller Gorge, New Zealand.

Yakovleva, M.N. (1963), Vop. Prikl. Radiogeol. 271-277, Effect of the age of various plant organs on the distribution of uranium in them.

Zlobin, V.S. (1966), Radiobiologiya 6 613-617, Accumulation of uranium and plutonium by seaweeds.

Zobel, C.R., Beer, M. (1961), J. cell. Biol. 10 335-346, Electron stains, I Chemical studies on the interaction of DNA with uranyl salts.

PUBLICATIONS ARISING FROM THIS THESIS

Brooks, R.R., Whitehead, N.E. (1968), J. scient. Instr. Ser. 2 (1) 879-881, A fluorimetric attachment for an atomic-absorption spectrophotometer.

Whitehead, N.E., Brooks, R.R. (1968), Econ. Geol. 64 50-56, A comparative evaluation of scintillometric, geochemical and biogeochemical methods of prospecting for uranium.

Whitehead, N.E., Brooks, R.R. (1969), J. appl. Ecol. 6 301-310 Radioecological observations on plants of the Lower Buller Gorge region of New Zealand and their significance for biogeochemical prospecting.

Whitehead, N.E., Brooks, R.R. (1969), Bryologist 72 501-507, Aquatic bryophytes as indicators of uranium mineralization.

Coote, G.E., Cohen, N.E., Whitehead, N.E. (1970), N.Z. Jl. Geol. Geophys. (in press), Investigation of uranium by high resolution gamma-ray spectrometry.

Whitehead, N.E., Brooks, R.R., Coote, G.E. (1970-71), Gamma radiation of some plants and soils from a uraniferous area in New Zealand. To be published in N.Z. Jl. Sci.

Whitehead, N.E., Brooks, R.R., Peterson, P.J. (1970-71), The nature of uranium occurrence in the leaves of *Coprosma australis* (A. Rich.) Robinson. To be published in Austral. J. biol. Sci.

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