OESOPHAGEAL CANCER IN THE TRANSKEI: MULTI-ELEMENTAL INVESTIGATION OF PLANT MATERIAL AND WATER

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1. INTRODUCTION

The present report forms a part of a large inter-disciplinary research programme, the study of the epidemiology of cancer of the oesophagus in selected geographical regions of Southern Africa. The programme was initiated when the striking demographic distribution of oesophageal cancer in Southern Africa first began to emerge, nearly two decades ago. [Burrell, 1962, Marais and Drewes, 1962]. Specifically, the Xhosa-speaking people of the Transkei region have the second highest rate of incidence of this cancer in the world. [Doll, 1969].

Although it is probable that no single factor is responsible for the aetiology of the disease, it is worthwhile to investigate possible environmental factors which could underline population differences in susceptibility to neoplastic disease. One of these factors, which is related to both the geographical location and to the technological development, is the trace element balance of the environment. This detailed balance, through the food and water chain, is reflected in the trace element balance of the human organism and may, either in excess or deficiency, be correlated with the disease. Although the ultimate deciphering of the extrinsic and intrinsic factors is likely to be a complex problem, this strategy, of investigating carcinogenic and/or susceptibility agents through epidemiologic studies is gaining increasing support from international research organisations (c.f. Payer, 1972).

In the Transkei, the content of trace metals in soils is highly variable in neighbouring regions for natural reasons, especially leaching of the upper surface. Although extensive studies of the relevant geographical pathology have been undertaken, no definite causal relationship has as yet been established (c.f. Harrington et al. 1975, and 1976).

The purpose of the present work is a quantitative, multi-elemental investigation of plant and food material in the Transkei. To ensure statistical reliability, a large sample suite from high and low incidence areas and from different geological regions has been chosen.

Specifically, since maize forms the staple diet of the region, leaf material from the maize plant (Zea mays) was selected for analysis (the leaf is generally a reliable indicator of the trace elemental composition of the soil and of the plant tissue itself; c.f. Peterson, 1972). In successive reports studies of other factors (especially water analysis and detailed geographical variations) will be reported. Similar studies are also under way in the Caspian Littoral of Iran, another region with high oesophageal cancer rates. [Kmet and Mahboubi, 1972]. In particular, trace elements in rice are determined by neutron activation analysis (c.f. Abedinzadeh, 1977).

In this report the protocol for the collection, handling and trace elemental analysis of the selected material is described, with special emphasis given to quality control. Contamination risks are carefully considered, in view of the long distances involved between the sampling region and the testing laboratory. In addition, since reliable sampling techniques are essential, careful attention was given to the choice of sampling position on the maize plant itself, and to the si and homogenization of the leaf samples.

The variation in the trace element concentration from place to place within the maize leaf must be sensitively known, so as to eliminate any spurious variables in the interpretation of the data obtained. Accordingly, a study on the longitudinal distribution of trace elements in the maize leaves was undertaken, to answer the fundamental question concerning the representative character of a single leaf section. This report summarises the results of this study, in a field where only very limited information exists.

2. ACCURACY AND QUALITY CONTROL; METHODS

Trace element analysis can be affected to a significant degree by contamination of the specimen during the process of collection, as well as by the contamination of reagents and of the environment during the analytical process. Accuracy and quality control must therefore cover all phases of the process, especially in the case of biological specimens where the elements of interest are present at extremely low concentrations.

In this work, a rigid protocol was established to minimise contamination (especially metallic) of the specimens. Particular attention was given to three aspects of quality control, based on the careful study of Boutwell (1976)

a) The validity (accuracy and specificity) of the analytical process for the designated element or elements of interest in the specimen matrix. In this work, two techniques have at this stage been employed: Instrumental Neutron Activation Analysis (INAA) and Particle Induced X-Ray Emission (PIXE).

b) The reliability of the sample collection, handling and preparation processes, and contamination control.

c) The accuracy of the calibration process with respect to the designated natural specimens and the analytical process.

Each of these aspects is discussed separately.

a) The analytical process

A major limitation to extensive trace element studies in plant samples has been the lack of rapid and practical methods for the measurement of a large group of elements in the ppm to ppb range. Traditional methods of analysis include wet

chemical techniques, spectrographic and flame photometric procedures.

In this work, we sought a method that would satisfy the following criteria:

(i) rapid and convenient;
(ii) little pre- or post-treatment of sample required;
(iii) capable of determining a large range of trace elements at low concentrations in a single sample;
(iv) non-destructive, i.e. allowing duplicate analyses on the same sample.

Accordingly, two methods were chosen as being most suitable: PIXE and INAA, and these are discussed in this section.

PIXE: The advantages of PIXE as a precise and rapid analytical method have been extensively reviewed (c.f. Johansson and Johansson, 1976). The potential of PIXE for the analysis of biological and medical samples is well known and is being increasingly utilized (c.f. Walter et al., 1977, and Kemp et al., 1975). More specifically, in the field of trace elemental studies in carcinogenesis, PIXE has found application and promises to be a powerful method (Guffey et al., 1977, Jundt et al., 1974).

The PIXE system at the University of the Witwatersrand is based on an EN Tandem Van de Graaff accelerator, a high vacuum target chamber and an Intrinsic Germanium X-ray spectrometer. Details of the experimental arrangement and capabilities of the system have already been reported (Annegarn et al., 1977). In this work, both whole and ashed leaf specimens were analysed. The following 9 elements were determined; K, Ca, Mn, Fe, Cu, Zn, Br, Sr, Rb.

This range of elements is in exact agreement with the results of Walter et al. (1977) who analysed several types of botanical samples, ranging from grass to plant and tree leaves. In all cases the same 9 elements were found, thus delineating the optimum range of sensitivity of PIXE. (In one exceptional case, Pb was also observed, which was attributed to the effects of automobile pollution near the roadside). In similar studies with animal samples, however, the same authors determined up to 18 elements, indicating the very low concentrations found in plant material (and/or the adverse detection limits of PIXE for these elements).

Detailed intercomparisons of PIXE with respect to other techniques are rather meagre and more studies of this type are warranted. Wheeler et al. (1974), reviewed the relative merits of PIXE, X-ray fluorescence and flame atomic absorption for biological specimens; PIXE was capable of determining the largest number of elemental concentrations simultaneously. The other methods have advantages in precision and simplicity of operation. Similar conclusions were reached by Camp et al. (1974). In the work of Florkowski et al. (1977), XRF was used to determine trace elements in plant material (mostly forest moss) for environmental pollution studies. On a routine basis, 7 elements could be determined; with special experimental arrangements, pollutants such as sulphur could be analysed as well.

In the present work, the same maize leaf samples studied by PIXE were also analysed by means of INAA, and 18 elements were determined. In the light of this, in conjunction with the studies cited above, the superior sensitivity of INAA for the analysis of plant material is firmly indicated. The discussion which follows thus concentrates on this technique alone.

INAA: Extensive investigations of biological and medical

samples have been undertaken with the aid of INAA, and the capabilities of the method are regularly being extended (c.f. Davies, 1976,). The wealth of applications of the method are clearly illustrated in the international symposia on activation analysis in the life sciences, such as those arranged every fourth year by the IAEA. In the specific field of the relationship of trace element concentrations and neoplasia, a good review of the validity of INAA has been provided by Zdankiewicz et al. (1976).

INAA Irradiation: All irradiations were carried out in the (nominally) 20MW Oak Ridge type research reactor of the South African Atomic Energy Board. All the samples and standards were irradiated in the pneumatic facility for 60 minutes in a predominantly thermal neutron flux of $\sim 1 \times 10^{17} n\ m^{-2}\ s^{-1}$. Westcott's epithermal index, r, for this irradiation position is 0,0087 (Turkstra et al., 1976). Relative values of the integrated neutron flux were determined for each sample and each reference material by the simultaneous activation of accurately weighed iron monitors ($\sim$ 0,030 g of iron) wrapped around each polythene ampoule. Reviews of the analytical procedures employed at the Nuclear Physics Research Unit are given by Fesq et al. (1973) and Erasmus et al. (1976).

Gamma Spectroscopy: In the present work, a 45 cm^3 Ge(Li) gamma spectrometer interfaced to an Intertechnique 4000 channel analyser was used for activity measurements. The resolution was 2,0 keV for the 1332 keV peak of Co-60, and the relative efficiency was 10%. The counting geometry was standardised and thus reproducible. Dead time corrections, which were usually less than 1% (and always less than 15%), were performed by means of a pulse generator as described by Anders (1969). The accumulated spectra were recorded on magnetic tape for subsequent analysis with a modified version of the Hevesey programme (1968). The radionuclides were identified by their

characteristic half-lives and gamma energies [Adams and Dams] (1970). A summary of the elements determined, photopeaks measured, decay and counting times, and the interfering nuclides (for which corrections were made) is given in Table 1. As expected, activities from the short-lived isotopes Na, K and Mn were high initially; levels were not as high as from animal samples, however, and interferences were not unduly severe.

b) Sample handling and preparation

All the leaf samples analysed were collected from field grown maize crops in the same district of the Transkei (Kentani - a high incidence area). Each leaf was taken from the same part of the maize plant, nearest the cob. Only mature plants were selected, at the tasseling stage with ripening cobs. Plastic gloves were used throughout. Immediately after collection, the leaves were double-sealed in polythene bags and deep frozen. Batches of leaves were airfreighted to the Wits laboratory in Johannesburg to await analysis; a low temperature was maintained throughout.

In the laboratory, sample preparations were carried out in an "ultra-clean", dust-free room, to ensure that both losses and contamination were kept to a minimum. Anand et al. (1975), for example, cautioned against contamination by routine laboratory procedure for trace element analysis, whilst a comprehensive review of the precautions necessary for the determination of meaningful values has been given by Mitchell (1973). Our procedure was devised taking due cognisance of these recommendations.

Three samples were taken, using a plastic knife, from each leaf; from the region nearest the stalk, from the middle section, and from the region nearest the tip. Each section included the midrib. These sections were dried at 85°C in a

drying oven for 24 hours, and then finely ground in an agate mortar. The sections were individually homogenised, further dried for 2 hours and then sealed in polythene ampoules. All materials were washed in distilled water.

All the biological reference materials (see next section) were also dried for 2 hours at 85°C before weighing and sealing, following the recommendations of the National Bureau of Standards. These procedures minimise the loss of important volatile elements (c.f. Donev and Marichkova, 1976, and Fourie and Peisach, 1977). All samples, both known and unknown, had a minimum mass of 500 mg. The problem of elemental retention after low temperature ashing has also been carefully discussed by Lutz et al. (1976). It was found that at temperatures below 100°C no significant losses occurred, even for elements such as arsenic, selenium and chromium, which are known to form thermolabile compounds.

c) Reference materials

Primarily two reference materials were used in this work to calibrate the analytical system: NBS tomato leaves (SRM 1573) and NBS spinach leaves (SRM 1570) [Cali, 1976]. Good results can be expected from this comparative technique, since the close similarity of the known and unknown sample matrices requires fewer corrections for nuclear interferences.

In addition, several other reference materials were employed, owing to the large number of elements determined, not all of which are certified by NBS. These materials, provided by the National Institute for Metallurgy, are part of the NIMROC set of international comparison standards; PTO-1, NIM-G, NIM-L and OG-382. Values used are those given by Erasmus et al. (1976). Precision of analysis was further improved by the use of single element solution standards prepared by us for Na, K, Mn and Au.

Based on these reference materials, a calibration curve was drawn for each element determined in the leaf samples. Fig. 1 shows the curves obtained for ^{140}La and ^{51}Cr; clearly the precision of determination is enhanced since the curve represents the average over a large number of determinations, over a wide range of concentrations and over differing decay times. It has the further advantage of indicating the effect, if any, of neutron and gamma self-shielding in the sample. These corrections were found to be negligible at the concentrations found in this work. In addition, each reactor irradiation included an activation of every reference material, which enabled the stability and and reliability of the analytical process to be monitored.

3. RESULTS

Table 2 displays the results obtained in our analysis of NBS tomato leaves, whilst those for spinach are given in table 3. Our mean values are observed to be in good agreement with those certified by NBS for the reference materials, whilst it can be seen from the table that values for several additional elements are suggested by the present work.

The new NBS reference material pine needles (SRM 1575) was also analysed, both as a check on our procedures, and as a contribution to the data being accumulated internationally on botanical reference samples. Values obtained are listed in table 4. It is clear that very good agreement is obtained with the NBS certified values for the 6 elements where such an inter-comparison is possible. Values for seven additional elements, not listed by NBS, are obtained in the present work; in addition, data for 5 elements, listed but not certified by NBS are determined by us, thus improving the international data set for these elements.

The values obtained for iron and chromium in the reference materials warrant special mention. From Table 2, it is clear that the results obtained in the present work for the botanical standard tomato leaves are in very good agreement with other published values and also with the data certified by NBS. However, the same is not true for spinach leaves, where the values obtained in the present work are seen to be rather higher than the certified data.

The problems of chromium determination are comprehensively discussed by Parr (1976). Results reported for analytical quality control samples issued by NBS and by IAEA over a number of years have consistently yielded poor results for chromium (with variations up to a factor of 35 for vegetable samples). Difficulties encountered include contamination (especially from stainless steel equipment), interfering nuclear reactions and loss of chromium during sample drying. In our work, in view of the high values obtained also for iron, it appears that some contamination has marred the integrity of the samples submitted for analysis. After a careful review of our procedures, and correction for Fe and Cr in the polythene blanks, it was concluded that this contamination could not have occurred in our laboratory, and was possibly introduced at an earlier stage of preparation. This suggestion is consistent with the results of Meloni et al. (1977) who obtained high values for Cr and Fe; see Table 3. However the results of Gallorini et al. (1976) are in good accordance with the certified values, indicating a non-homogeneous contamination.

In Tables 6 (Kentani), 7 (Bizana) and 8 (Butterworth), the analysis of maize leaves and the variation of (trace) elemental concentrations longitudinally across the maize leaves is given. A detailed examination of the results suggests that maize leaves cannot be regarded as homogenous; indeed factors of 2 difference from stalk to tip are found. However, trends in these

variations are not constant from district to district, and a strict interpretation of the intrinsic errors, leads to the more conservative conclusion that there are indeed fluctuations in concentrations across the maize leaf, but that these are not consistent. Sometimes inverse sympathetic response is seen (Na vs K, e.g.) but this is not always maintained. Hence an extensive study might well reveal overall patterns in such elemental distributions, but is, in our view, at this stage of lesser importance than seeking gross structure between high and low cancer incidence areas. Our deduction from this study is that a well-mixed sample obtained from the whole leaf should be studied to obtain a meaningful average for the leaf.

4. OVERVIEW OF PROJECT, AND FUTURE PROGRAMME

The overall objectives of the present work were as follows:

a) To seek to establish, if such exist, differences in trace elemental patterns between high and low oesophagal cancer areas (4 areas) in the Transkei, in selected relevant materials.

b) The selection and testing of a valid and sensitive nuclear technique (instrumental neutron activation analysis) for the quantitative determination of a broad range of trace elements in plant material (maize leaf). Subsequent studies will include specific elements thought to have carcinogenic relevance. Methods which are considered valid for investigation are short-lived neutron activation analysis, epithermal neutron activation (with or without radiochemical separation), and selected charged particle nuclear reactions. X-ray fluorescence may be added for major elements.

c) The establishment of a rigid protocol, with careful

quality control, for the collection, handling and treatment of the sampled material.

d) The investigation of the longitudinal distribution of trace elements in maize leaves.

e) The quantitative determination of elemental concentrations in NBS reference materials, as a contribution to the international data pool on these elements.

f) The analysis of the full suite of maize leaves.

g) The analysis of drinking waters for the same four areas.

It is believed that substantial progress has been made with these objectives and that continuing work in extending the overall programme to demographic and epidemographic studies (based on the techniques discussed in this paper) remains warranted.

With regard to objective a), this remains still valid.

Objective b) has described much of our endeavours during this year. Considered with objective e), the method of instrumental neutron activation analysis (INAA) has been thoroughly developed for this purpose and is now in routine use. This will be expanded shortly by the exploration of short-lived INAA and of epithermal INAA. We are engaged in both paper-studies and exploratory research in charged particle methods - Table 9 reflects the possibilities for the analysis of such elements as have not been seen by INAA. While our INAA measurements will continue (now routinely) on the maize leaf samples this year, research will continue on these complementary nuclear techniques, with hopefully some routine application later in the year. Considering objective e) more specifically, the present work determines some 18 elements in three NBS botanical reference materials. In tomato leaves, 5 additional elements (Na, Cs, Ba, Sm and Ta) are evaluated, besides those

certified by NBS; in spinach 6 additional elements are quantified (also Au) and in pine needles we give an extra seven values (also Zn). Since these reference materials are of considerable importance in the assay of vegetable and food samples, we believe that the present work provides valuable additional data in the field of multi-elemental analysis. We confirm that the INAA technique combines the advantages of sensitivity, non-destructiveness and a broad elemental range, especially if sub-ppm amounts are to be determined.

Objective c) has been achieved.

With respect to objective d), several studies have been performed concerning the effect of trace element concentrations on plant growth. However, data regarding critical levels in plants are only partially complete, and only very limited information exists pertaining to the distribution and mobility of trace elements within the tissue of plant leaves. In the study of Vose et al. (1961), the method of XRF was used to study plant material, and the questions of homogeneity were quantitatively discussed, although no localisation of elements was attempted. Neutron activation was used by Haller et al. (1969) in a multi-elemental investigation of plant material; the emphasis was on development of the technique and again no distributional studies were attempted. The availability of trace elements to plants, and the soil-plant interaction were discussed by Hemphill (1972). Average levels of minerals important to plant growth were determined, and factors affecting their uptake (e.g. availability, fertilization of soils, acidity) were discussed. It was found that fruit trees are good indicators of soil concentrations, and that of all the field and vegetable crops, maize (corn) is the most sensitive. The accumulation and distribution of elements in plants was investigated by Peterson (1972); each

plant species was divided into broad categories; root, twigs, leaves and concentrations determined in each. No detailed studies of distributions within foliage were undertaken. A similar investigation was carried out by Cannon et al. (1972) who also studied seasonal variations of trace elements in vegetation. More recently, a limited investigation of trace elemental abundances over the cross section of a leaf was carried out by Walter et al. (1977). A single oak leaf was scanned for uniformity by means of PIXE; results were reported for variations in 9 elements. The majority of elements were non-uniform by 20% to 30%, although some varied by up to 40% (range). No systematic pattern could be discerned, however, either laterally or longitudinally, confirming our result that there are fluctuations, but no specific distribution patterns.

With regard to objective f) it is believed that the present work already provides a useful supplement to the rather meagre amount of multi-elemental data on plant composition thus far available. In Table 6, "normal" values of trace concentrations in maize leaves are listed (with references). As far as is known, reliable data for comparison exist only for 7 elements; nevertheless important conclusions can be drawn. It is clear that values determined in Transkei maize leaves for elements such as Co, Ca, Cr and Fe are high; this is particularly the case for Co, which is higher than the optimum value by a factor of up to 20 (see Table 5). In the absence of documented values, it is unclear whether this should be regarded as a toxic level (to the plant) or not. Elements such as Zn and Mn fall within the range of acceptance for the plant (defined as being neither toxic nor deficient, c.f. Chapman 1965).

The values found for potassium indicate that trace levels in the Transkei are on the margin of deficiency. This is

particularly true for values determined in the tips of the leaves. The longitudinal distribution of potassium in the leaves follows the classic K deficiency pattern described by Chapman (1965) where potassium ions are removed first from the tips and edges of leaves and transported to the rapidly growing cob.

The advantages of using plant leaves as reliable indicators of plant nutrient status and thus as sensitive monitors of trace levels in soils (as suggested by Tyner, 1946) are thus effectively endorsed by the present work. We conclude that the trace elemental concentrations in leaf samples taken at the bloom stage are a good measure of the relative nutrient reserves present in the plant early in the reproductive stage, a critical period for all varieties. Further, the present work provides quantitative data for 11 elements present but not as yet documented for maize, an important food crop.

However, it is appropriate at this stage to take stock of our position. Table 10 reflects the inventory of maize leaves collected and held by ourselves. To date we have irradiated 54 samples - following the same philosophy, there are 1518 analyses still to be done! Since the distribution of elements as a function of position on the leaf is, as we have shown, dominated by fluctuations without consistent trends, and since we believe it to be important to expand the number of elements measured by complementary nuclear techniques as described earlier, we propose the following amended procedure;

- select one maize leaf from each package (retaining the remaining 3 for possible future use), and combine these single leaves for each of the 4 districts. For each district, these leaves will be dried, powdered and thoroughly mixed, providing therefore 4 samples truly representative of Kentani, Bizana, Butterworth and Lusikisiki respectively. From

these 4 "master samples", aliquots will be taken for each of the different nuclear techniques used. Should the results justify closer investigation, 3/4 of the material is still available in unmixed form for the purpose.

With regard to objective g), the analysis of water, we can report that a suite of 90 samples has been collected from the 4 districts. To date exploratory research has been carried out on methods. Four samples (one from each district) have been filtered through millipore filters, and the filter with the particulate material divided into two parts, one used for INAA and the other for PIXE. First preliminary results are given in Table 11. The filtrate has not yet been analyzed, but considerable effort has gone into methods for pre-concentration of trace elements. These will now be applied, both for INAA and for PIXE. Should satisfactory results be obtained, routine analysis (again of lumped samples initially) will follow. In parallel we have carried out research on the preparation of these water samples for nuclear analysis by lyophilization - this has been only partially satisfactory and this approach has been suspended until the arrival of a freeze drier (in March).

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TABLE 1

ISOTOPES USED IN THE ANALYSIS OF PLANT LEAVES

Element	Isotope observed	Half-Life	α Peak Energy KeV	Time elapsed (after irradiation) Days				Interference	Peaks not used due to Interference
				1.0	7.0	21.0	46.0		
Na %	Na-24	1.5hrs	1731.6 1368.4	*					
K	K-42	12.5hrs	1524.7	*					
Ca	Ca-47	4.5d	1296.9 160.0	*	*			Sc-47	160.0
Mn	Mn-56	2.6hrs	1810.7 846.9	*	*				
Fe	Fe-159	45d	1291.5 1098.6		*	*	*	Ta-182	
Sc ppm	Sc-46	84d	1120.3 889.4			*	*	Ta-182	1120.3
Cr	Cr-51	27.8d	320.0		*	*	*		
Co	Co-60	5.25yrs	1332.4 1173.1		*	*	*		
Zn	Zn-65	243.8d	1115.5		*	*	*	Ta-182	
As	As-76	1.1d	559.8	*					
Br	Br-82	1.48d	776.6 554.3	*	*				
Rb	Rb-86	18.7d	1076.6			*	*		
Sr	Sr-87m	2.8hrs	388.4	*					
Sb	Sb-124	60.0d	1690.6				*		
Cs	Cs-134	2.05yrs	795.8 604.7			*	*		604.7
Ba	Ba-131	12.0d	496.5 216.0		*	*			
La	La-140	1.68d	1596.2 486.8	*	*				
Sm	Sm-153	1.95d	103.2 69.6	*	*				
Ta	Ta-182	115d	1221.6 1121.2		*	*	*	As-76	1121.2
Au	Au-198	2.7d	411.8	*	*	*		Eu-152	

Counting time for 1st Count = 30 minutes
2nd " = 60 minutes
3rd " = 120 minutes
4th " = 120 minutes

All data normalised to;- 1 hour counting time
2cm counting distance

TABLE 2

ANALYSIS OF NBS TOMATO LEAVES (3 ANALYSES)

Element	Present Work Value in ppm or %	S.D.	Nbs Std values	Quoted Uncertainties	Ref37 Guzzi 1976 Mean Value	±5%	Ref37 R Stell et Mean Value	±5
Na %	0.05	±0.02	-	-				
P	-	-	0.34	±0.02				
K	4.15	±0.08	4.46	±0.03				
Ca	2.4	±0.07	3.0	±0.03				
Mn	0.02	±0.0004	0.02	±0.0007	0.021	±0.97		
Fe	0.073	±0.009	0.069	±0.0025	0.0507	±2.81		
Sc ppm	0.16	±0.03	(0.13)		0.138	±5.08		
Cr	4.5	±1.6	4.5	±0.5				
Co	0.68	±0.03	(0.6)		0.467	±5.33		
Cu	-	-	11	±1.0				
Zn	62.0	±4.6	62.0	±6.0				
As	-	-	0.27	±0.05				
Br	21.0	±3.0	(26.0)				25.31	±1.(
Rb	15.21	±2.3	16.5	±0.1	16.4	±3.21		
Sr	-	-	44.9	0.3				
Sb	0.12	±0.05	(0.04)					
Cs	0.14	±0.03	-					
Ba	69.0	±14.0						
La	0.8	±0.2	(0.9)					
Sm	0.2	±0.09	-					
Ta	0.43	±0.30	-					
Pb			6.3	±0.3				
Th			0.17	±0.03				
U			0.061	±0.003				

Note: ( ) Non-Certified values

TABLE 3

ANALYSIS OF NBS SPINACH

Element	Present Work Value in ppm or %	C.D.	Nbs Std Values	Quoted Uncertainties	Ref.36 Meloni et al 1977 Values	Ref.38 Gallorini et al 1976 Values	
Na %	1.54	±0.14	-	-			
Al	-	-	0.087	±0.005			
P	-	-	0.55	±0.02			
K	3.26	±0.23	3.56	±0.03			
Ca	1.78	±0.25	1.35	±0.03			
Mn	0.02	±0.0004	0.02	±0.0006		0.0118	±0.003
Fe	0.066	±0.03	0.055	±0.002	0.0763		
Sc ppm	0.18	±0.02	(0.16)		0.47		
Cr	7.5	±1.6	4.6	±0.3	21.0	5.2	±0.5
Co	1.5	±0.2	(1.5)		1.68	1.7	±0.1
Cu	-	-	12.0	±6.0			
Zu	47.0	±0.48	50.0	±2.0	57.0	72.5	±1.6
As	-	-	0.15	±0.05			
Br	48.0	±9.4	(54.0)				
Rb	11.32	±3.1	12.1	±0.2	17.0		
Sr	-	-	87.0	±2.0			
Sb	0.69	±0.15	(0.04)				
Cs	0.27	±0.04	-				
Ba	87.0	±29.0	-				
La	0.35	± 0.06	(0.37)				
Sm	0.20	±0.14	-				
Ta	0.23	±0.08	-				
Au	0.002	±0.0004	-				
Hg			0.030	±0.0005			
Pb			1.2	±0.2			
Th			0.12	±0.03			
U			0.046	±0.0009			

Note: ( ) Non-certified values

TABLE 4

ANALYSIS OF NBS PINE NEEDLES

Element	Value in ppm or %	S.D.	NBS Std Values	Quoted Uncertainties
Na %	0.0045	±0.0007	-	
Al			0.0545	±0.003
P			0.12	±0.02
K	0.37	±0.02	0.37	±0.02
Ca	0.43	±0.06	0.41	±0.02
Mn	0.07	±0.01	0.0675	±0.0015
Fe	0.028	±0.005	0.02	±0.001
Mn-54	0.50	±0.15		
Sc ppm	0.053	±0.008	(0.03)	
Cr	3.93	±0.05	2.6	±0.2
Co	0.34	±0.18	(0.1)	
Cu			3.0	±0.3
Zn	65.0	±6.0	-	
As			0.21	±0.04
Br	6.1	±0.09	(9)	
Rb	12.5	±3.9	11.7	±0.1
Sr			4.8	±0.2
Sb	1.14	±0.44	(0.2)	
Cs	0.16	±0.06	-	-
La	0.21	±0.03	(0.2)	
Sm	0.13	±0.12	-	
Ta	1.74	±0.27	-	
Au	0.0003	±0.00008		
Hg			0.15	±0.05
Pb			10.8	±0.5
Th			0.037	±0.003
U			0.020	±0.004

Note;- ( ) Non certified values

TABLE 3

ANALYSIS OF NBS ORCHARD LEAVES (2 ANALYSES)

Element	Present Work Value in ppm or %	S.D.	NBS Std Values	Quoted Uncertainties
Be %			0.000003	±0.0000001
B			0.0033	±0.0003
N			2.76	±0.05
Na	0.012	±0.004	0.0082	±0.0006
Mg			0.62	±0.02
P			0.21	±0.01
K	1.47	±0.10	1.47	±0.03
Ca	1.90	±0.11	2.09	±0.03
Mn	0.011	±0	0.0091	±0.004
Fe	0.045	±0.007	0.03	±0.2
Sc ppm	0.080	±0.0057		
Cr	5.81	±0.84	2.6	±0.3
Co	0.46	±0.10	(0.2)	
Ni			1.3	±0.2
Cu			12.0	±1.0
Zn	29.63	±1.80	25.0	±3.0
As	8.66	±1.25	10.0	±2.0
Se			0.08	±0.01
Br	9.19	±1.39	(10.0)	
Rb	15.61	±3.01	12.0	±1.0
Sr			(37)	
Mo			0.3	±0.1
Cd			0.11	±0.01
Sb	2.57	±0.19	2.9	±0.3
Cs	0.15	±0.06	(0.04)	
Ba	59.54	±1.81	(44)	
La	1.24	±0.08		
Sm	0.32	±0.12		
Au	0.00078	±0.00015		
Hg			0.155	±0.015
Pb			45.0	±3.0
U			0.029	±0.005

Note: (Non-certified values)

TABLE 6

VARIATION OF TRACE ELEMENTS ALONG THE LENGTH OF MAIZE LEAVES

(6 ANALYSES) KENTANI -- HIGH INCIDENCE AREA

CONCENTRATION IN % AND IN PPM

ERRORS ARE STANDARD DEVIATIONS

	Area near Tip		Midsection		Stalk area	
Element	Value	S.D.	Value	S.D.	Value	S.D.
Na %	0.12	±0.04	0.10	±0.05	0.06	±0.03
K	1.28	±0.30	1.69	±0.75	2.29	±0.81
Ca	1.17	±0.37	0.82	±0.22	0.64	±0.14
Mn	0.007	±0.003	0.003	±0.001	0.003	±0.001
Fe	0.05	±0.02	0.08	±0.03	0.05	±0.02
Sc ppm	0.05	±0.02	0.04	±0.01	0.10	±0.06
Cr	10.7	±2.7	10.5	±1.9	7.5	±2.4
Co	1.30	±0.34	1.33	±0.58	1.05	±0.39
Zn	41.4	±8.9	43.4	±14.8	52.1	±15.0
Br	94.1	±42.9	85.9	±36.6	142.8	±60.6
Sb	-	-	0.09	±0.01	0.48	±0.15
Cs	0.45	±0.07	0.40	±0.07	0.60	±0.6
La	0.17	±0.04	0.18	±0.04	0.16	±0.06
Sm	-	-	-	-	0.12	±0.09
Ta	1.22	±0.59	1.95	±0.13	0.91	±0.61
Au	0.003	±0.001	0.002	±0.001	0.004	±0.002

TABLE 7

VARIATION OF TRACE ELEMENTS ALONG THE LENGTH OF MAIZE LEAVES

(4 ANALYSES) BIZANA - LOW INCIDENCE AREA

CONCENTRATION IN % AND IN PPM

ERRORS ARE STANDARD DEVIATIONS

	Area near Tip		Midsection		Stalk Area	
Element	Value	S.D.	Value	S.D.	Value	S.D.
Na %	0.07	±0.03	0.04	±0.0007	0.02	±0.005
K	1.73	±0.42	1.38	±0.29	1.48	±0.72
Mn	0.007	±0.003	0.009	±0.002	0.006	±0.002
Mn-54	3.65	±0.10	0.95	±0.06	0.68	±0.22
Sc ppm	0.07	±0.02	0.04	±0.01	0.06	±0.02
Cr	12.5	±4.1	9.08	±2.21	7.3	±3.01
Co	1.07	±0.57	0.67	±0.13	0.59	±0.21
Zn	33.9	±4.6	40.0	±7.6	32.5	±4.8
Br	49.0	±9.5	19.5	±6.6	39.7	±22.5
Rb	33.9	±10.2	41.2	±10.7	15.9	±1.5
Cs	0.47	±0.13	0.43	±0.08	0.26	±0.05
La	0.30	±0.13	0.21	±0.11	0.16	±0.04
Sm	-	-	-	-	0.05	±0.01
Au	0.02	±0.0002	0.001	±0.002	0.001	±0.0003

TABLE 5

VARIATION OF TRACE ELEMENTS ALONG THE LENGTH OF MAIZE LEAVES

BUTTERWORTH - HIGH INCIDENCE AREA

(4 ANALYSES)

CONCENTRATION IN % AND IN PPM - ERRORS ARE STANDARD DEVIATION

	Area near Tip		Midsection		Stalk area	
Element	Value	S.D.	Value	S.D.	Value	S.D.
Na %	0.057	±0.04	0.043	±0.02	0.037	±0.007
K	1.7	±0.55	2.20	±0.005	1.98	±0.65
Mn	0.023	±0.017	0.011	±0.005	0.0098	±0.0004
Fe	0.083	±0.02	0.073	±0.02	-	-
Sc ppm	0.061	±0.022	0.062	±0.009	0.058	±0.008
Cr	17.08	±2.62	27.94	±5.65	9.54	±2.97
Co	2.02	±0.40	2.01	±0.88	0.99	±0.28
Zn	47.90	±13.81	41.23	±12.58	41.19	±6.09
Br	72.51	±47.07	65.09	±36.99	119.05	±61.15
Sb	-	-	1.29	±0.13	-	-
Cs	0.61	±0.07	0.91	±0.44	0.74	±0.48
La	1.01	±4.3	0.71	±0.22	0.63	±0.05
Sm	0.68	±0.64	0.31	±0.27	0.57	±0.02
Ta	1.16	±0.45	-	-	-	-
Au	0.0029	±0.0016	0.0037	±0.0016	0.0023	±0.0013

TABLE 2

I. Essential elements not seen by long-lived INAA

P	:	CPpγ
Cl	:	CPAA ($\tau_{\frac{1}{2}}$ = 2.50m); CPpγ
Cr	:	PIXE, RNAA
Se	:	RNAA; CPAA ($\tau_{\frac{1}{2}}$ = 56h)
Mo	:	ENAA
Sn	:	(CPpγ)
I	:	RNAA

II. Beneficial elements not seen by long-lived INAA:

Li	:	CPβ$^-$
V	:	S-LNAA
Ni	:	ENAA; CPAA($\tau_{\frac{1}{2}}$ = 3.41 h); CPpγ

III Biologically active elements not seen by long-lived INAA:

Be	:	CPA; CPpγ
As	:	S-LNAA
Cd	:	RNAA
Hg	:	RNAA
Pb	:	(CPA?); (PIXE?)

IV "Other" elements not seen by long-lived INAA:

B	:	CPβ$^-$; CPAA ($\tau_{\frac{1}{2}}$ = 52 d)
C	:	CPβ$^+$ CPpγ
N	:	CPβ$^+$ FNAA
Mg	:	S-LNAA
Al	:	S-LNAA
P	:	ENAA(S-L)
S	:	Pre-separation + S-LNAA; CPpγ
Cl	:	S-LNAA; CPpγ
Ba	:	(Very long irradiation INAA?)

Code:

CPpγ	=	charged particle prompt γ
CPAA	=	charged particle activation analysis (γ)
RNAA	=	neutron activation analysis and radiochemical separation
PIXE	=	proton induced X-ray emission
ENAA	=	epithermal neutron activation analysis
CPβ$^+$	=	charged particle induced β$^+$ decay
CPβ$^-$	=	charged particle induced β$^-$ decay
S-LNAA	=	short-lived neutron activation analysis
CPA	=	charged particle analysis (particle detection)
FNAA	=	fast neutron activation analysis
(S-L)	=	short-lived

TABLE 10

INVENTORY OF MAIZE LEAVES

District	Region	No. of packets	No. leaves per packet	INAA analysis	
				No. packets	No. leaves ex packet
Kentani (High cancer)	Upper	23	4	1	2
	Lower	24	4	1	4
Bizana (Low cancer)	Upper	13	4	1	4
	Lower	13	4	-	-
Butterworth (High cancer)	Upper	13	4	1	4
	Lower	13	4	-	-
Lusikisiki (Low cancer)	Upper	16	4	1	4*
	Lower	16	4	-	-
				Total of leaves analyzed	18

(3 positions on each, therefore 54 samples analyzed)

* Now being analyzed

TABLE 11

ANALYSIS OF TRANSKEI WATER

NEUTRON ACTIVATION ANALYSIS

CONCENTRATION IN PPM OR PPB

ERRORS ARE STANDARD DEVIATIONS

Element	Butterworth #72		Kentani #88		Bizana #45		Lusikiki #64	
	Value	S.D.	Value	S.D.	Value	S.D.	Value	S.D.
Na ppm	0.0052	± 0.003	0.034	±0.032	0.0066	± 0.0005	0.0042	±0.0005
Ca					2.24	± 0.89		
Sc ppb			0.36	±0.14				
Cr	44.88	±12.42			23.96	±10.44		
Co	3.19	± 0.79	1.08	±0.05	0.82	± 0.59	1.82	±1.48
Zu					131.0	± 0.01		
Br	4.96	± 1.73	5.87	±1.17	4.24	± 1.48	4.49	±0.73
La	0.76	± 0.29	0.51	±0.15			0.29	±0.09
Sm	2.04	± 0.56	3.93	±0.08	3.35	± 0.47	1.02	±0.25
Au			0.0027	±0.001	0.013	± 0.001		

PIXE

ERRORS ARE PERCENTAGES

	Value	Error %	Value	Error %	Value	Error %	Value	Error %
Ti ppb	59.0	2.5	31.4	4.0	3.53	35.0	1.60	68.0
Cr	37.0	2.8	<0.5	100.0	1.53	45.0	8.60	7.0
Mn	7.60	>7.0	16.9	5.6	29.8	2.8	65.8	1.4
Fe	219.0	0.7	430.0	0.5	208.0	0.7	146.0	0.8
Ni	4.77	10.5	0.3	100.0	1.16	29.0	0.90	31.0
Cu	4.75	9.5	4.04	12.6	2.61	17.0	1.77	20.0
Zn	52.3	2.0	72.0	1.7	109.9	13.0	24.4	3.0
As								
Pb			<1.7	100.0	<1.5	100.0		