ANALYSIS OF NUTRITIONALLY IMPORTANT ELEMENTS IN SOME VEGETABLES AND BIOLOGICAL STANDARD REFERENCE MATERIALS

A

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By

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled, "**ANALYSIS OF NUTRITIONALLY IMPORTANT ELEMENTS IN SOME VEGETABLES AND BIOLOGICAL STANDARD REFERENCE MATERIALS**" in partial fulfilment of the requirement for the award of the **Degree of Master of Philosophy** submitted in the Department of Chemistry of the University is an authentic record of my own work carried out during a period from January 1998 **to July 1998** under the supervision of **Prof. A.N. Garg.**

The matter embodied in this thesis has not been submitted by me for the award of any other degree.

Manjul Singh.

Date: 31st JULY, '98.

(MANJUL SINGH)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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ABSTRACT

Seventeen green and leafy vegetables have been analysed for eight nutritionally important elements (Ca, Cu, Mn, Mg, Fe, Co, Zn and Cr) and three environmentally toxic heavy metal pollutants (Ni, Cd and Pb) in various vegetables vary in a wide range. Average moisture and ash contents were found to be 88.4 and 9.65 % respectively for most samples. In addition, five botanical Standard Reference Materials, Bowen's Kale, spinach (CRM-331), cabbage (CRM-359), citrus leaves (SRM-1572) and tomato leaves (SRM 1572) were also analysed for quality control. Two samples of spinach and mint leaves were also analysed for 21 elements by neutron activation analysis and prompt gamma ray analysis. Room temperature Mossbauer spectrum of spinach exhibited two lines with small quadrupole splitting suggesting Fe^{3+} in high spin state.

Besides, a brief account of essentiality of the elements and their importance in human diet is and a attempt has been made to estimate intake of some essential elements through daily consumption of vegetables.

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INTRODUCTION

CHAPTER I

INTRODUCTION : TRACE ELEMENTS AND NUTRITION

I.1 NUTRIENTS AND THEIR FUNCTION

Man needs a wide range of nutrients for proper functioning of the body and to lead a healthy life. The nutrients include proteins, fats, carbohydrates, vitamins and minerals. Sometimes proteins, fats and carbohydrates are referred to as proximate principals, and are oxidized to yield energy needed by the body [1]. On the other hand vitamins and minerals do not supply energy but they play an important role in the regulation of the metabolic activity in the body and help in the utilization of the proximate principals. Minerals are also used for the formation of body structure and skeleton. In the following lines are described the role of various nutrients in living organisms.

(i) Proteins : These are vital to all living organisms and are the important constituent of tissues and body cells. They form an important component of muscle and other tissues including blood. The proteins in the form of enzymes and hormones are concerned with a wide range of vital metabolic processes in the body. These supply building material and make good the loss due to wear and tear of the body. The dietary proteins are broken down into amino acids absorbed as such and these amino acids are used by the body to synthesize the proteins needed by the body for various functions like tissue building, replacement of protein depleted and synthesize functional molecules like enzymes, hormones and antibodies.

(ii) Fats : It is an important component of diet and serves a number of functions in the body. Fat is a concentrated source of energy and it supplies per unit weight more than twice the energy furnished by either proteins or carbohydrates. It also imparts palatability of a diet and

retards stomach emptying time. Presence of fat in the diet is important for the absorption of fat soluble vitamins like Vitamin A. Some fats, derived from vegetable sources provide "essential fatty acids" (EFA) which have vitamin like functions in the body.

(iii) Carbohydrates : These are energy yielding substances which include starch, glucose, cane sugar, milk, sugar etc. These are primarily derived from cereals which form chief source of energy in Indian diet. Starch when eaten in cooked form is completely digested in the gastrointestinal tract and the released glucose is absorbed and metabolised in the body to yield energy. Many foods contain non-digestible carbohydrates like cellulose, hemicellulose, gums, pectins and lignins designated as "dietary fibre"

(iv) Energy : It is essential for rest, activity and growth. It is well known that even when a body is at rest, it expends certain amount of energy for essential functions including maintenance of body temperature. The amount of energy thus

expended when the body is at complete rest (both mentally and physically) is termed *Basal or Resting Metabolism*. The energy required for both basal metabolism and muscular activity have to be supplied through food.

(v) Vitamins : These are organic substances present in small amount in many foods. They are required for carrying out many vital functions of the body and many of them are involved in the utilization of the major nutrients like proteins, fat and carbohydrates. These can be broadly classified as water soluble and fat soluble vitamins. B-complex vitamins and ascorbic acid belong to the former group while vitamin A, D, E, K are fat soluble.

(vi) Minerals and Trace Metals : A large number of minerals and trace metals are present in the body. Some of these form part of body structural components and some others act as catalytic agents in many biochemical processes. Bones and skeleton are made up mainly of calcium,

magnesium and phosphorus, and iron is a component of blood. A classification of various elements required in human metabolism and found in varying concentrations is given in Table I.1.

I.2 TRACE ELEMENTS IN HUMAN METABOLISM

The bulk of the human body is composed of twelve major and minor elements - C, H, , O, Cl, Ca, P, Na, K, Mg, S and Si - which constitute 99.8% of the total body weight. Besides several trace elements such as Co, Cr, Cu, Fe, Se, Zn etc., have also been recognized as essential for various life processes [2].

Animals including man procure the elemental nutrients from food and water, as the direct intake of elements is not possible, if taken they are not easily absorbed by the system. Therefore, the best way to get the elemental nutrients is from the food we take. The food contains the elements in bioavailable form so they can be easily assimilated by the system. The food includes many elements that are

Table 1.1 Classification of elements important in the human metabolism

Structural elements (Occurrence > 1%)	C, Ca(hard tissues only), H, N,O,P,S
Electrolyte elements (Occurrence 0.01-1%)	Ca,Cl,K,Mg,Na and $(HCO_3)^-$,$(SO_4)^{2-}$ $H(HPO_4)^{2-}$
Trace elements (Occurrence < 0.01 %)	
1. Essential	
a. Biologically important	
Group I	Co, Cr, Cu, Fe, I, Mn, Mo, Sc, Zn
Group II	As, F, Ni, Si, Sn, V
b. Clinically relevant	(Cr), Cu, Fe, I, Se, Zn
2. Toxic	
a. Potentially toxic	As, Be, Cd, Hg, Pb, Sc, Tl
b. Major environmental contaminants	Cd, Hg., Pb
c. Industrial hazards	As, Be, Cr, Hg, Mn, Ni, Pb, Sb, Si
Other relevant elements	Ag, Al, Au, B, Ba, Bi, Br, Ce, Cs, Ga, Ge, Lki, Np, Pt, Rare earths, Rb, Sc, Sr, Te, Th, Ti, U, W, Zr.

nutritionally important like - Ca, Mg, P, Na, K, Fe, Cu, Ni, B, Zn, Cr, F etc, and it also contains some elements as the environmental contaminants, Cd, Hg, As, Br, Pb etc. A large number of elements are present in macro, micro and trace amounts in the body. If the intake of these elements is not sufficient then it can lead to malnutrition and structural and physiological abnormality. A chronological order of the establishment of essentiality of trace elements and their normal metabolic functions including deficiency symptoms are listed in Table I.2. All these elements, generally have an optimum range of concentration below which deficiency symptoms are observed and beyond which harmful and toxic effects are manifested as illustrated in Fig. I.1.

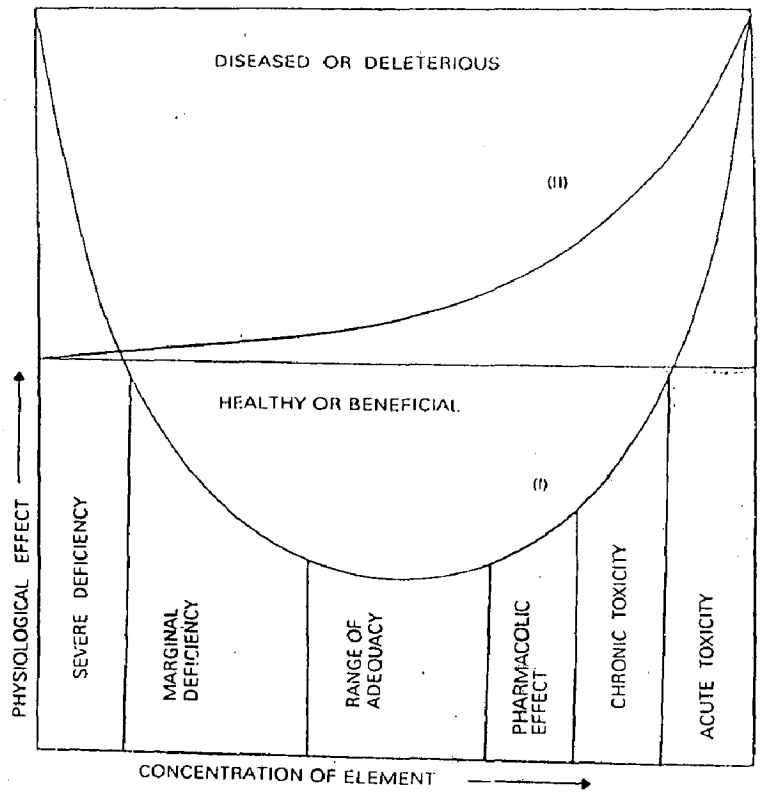

Fig. I. 1 Correlation of Elemental Concentration with its Physiological Effects

Table 1.2 : List of essential elements, their metabolic functions and deficiency symptoms

Element	Function	Deficiency Symptoms
Fe	Oxygen & electron transpsort, constitutes haemoglobin	Anaemia, Stomatitis, Dysphagia, brittle nails
I	Constituent of thyroid hormones	Goitre, depression of thyroid function, cretinism, dry hair, hardening of arteries
Cu	Constituent of oxidative ensymes, interacstion with iron, cross linking of elastin	Anemia, Change of classification, Elevated serum Cholesterol, diarrhea, nervous system damage
Mn	Mucopolysaccharide metabolism, constituent of superoxide dismutase	Disturbances in bones and cartrilege formatio, Deafness, Dizziness
Zn	Constituent of enzymes involved in energy metabolism and transcription	Growth depression, poor healing of wounds sexual immaturity, skin lesions, menstrual prolems, change of taste, acuity
Co	Constituent of Vitamin B_{12}	Loss of apetite, Vitamin B_{12} deficiency
Mo	Constituent of Xanthine, aldehyde and sulfide oxidases	Human dental caries, Male impotence
Sc	Constituent of glutahione peroxidase, interaction with heavy metals	Endemic cardiomyopathy, (Keshan disease) Premature aging, eye and nerve disorders, infertility
Cr	Potentiation of insulin	Relative insulin resistance, impaired glucose tolerance, elevated serum lipids, Fatigue and lack of energy, indigestion

Table I.2 (Contd.)

Element	Function	Deficiency Symptoms
Sn	Unconfirmed Reports	Possible growth depression
F	Structure of teeth bones, possibly growth effects	Increased incidence of caries, possible risk of osteoporosis
As	DNA repair mechanism	Growth depression
V	Unconfirmed Reports	Possible growth depression
Si	Calcification, role in connective tissue	Possible metabolic changes
Ni	Interaction with iron absorption, activates enzymes	Anaemia, in consistent growth
Ca	Essential for formation of healthy bones & teeth, regulates blood clooting, muscle function, nerve transmission	Muscle cramps, brittle bone disease, dental problems
Mg	Help in absorption of other minerals, simulates bone growth and promotes the body's use of vitamin B,C and E.	Lack of energy, muscle spasms, weakness, Asthma, Cardiovascular disorders
K	Essential for growth, simulates nerve impulses, promotes healthy skin, Boosts kidney functio, combines with Na to regulate heart beats.	Irregular heart beats, dry skin, nervous disorder
P	Cell repairs, vital to growth of bones and teeth, helps digest proteins, fats and carbohydrates	Poor growth, arthritis, loss of apetite.

(i) Action of Trace Elements : Metal ions play a vital role in a large number of widely differing biological processes some of which are quite specific in their metal ion requirements. The functional forms of trace elements and their characteristic concentration must be maintained within a narrow limit if the functional and structural integrity of the tissue is to be safeguarded and growth, health and fertility are to remain unimpaired [3]. They function primarily as catalyst in enzyme systems in the cells producing large biological effects. Their role may range from weak, ionic strength effects to highly specific associations known as metalloenzymes which constitute 30% of all known enzymes [3,4]. If the metals are removed from the metalloenzymes, there occurs a severe damage to physiological and metabolic functions. There are also a large group of enzyme systems called metal-ion activated coenzymes whose function is to induce or maintain the active conformation state of the protein molecule [3,4].

(ii) Need and Tolerances of Trace Elements : The minimum requirements of man for the essential trace elements are commonly expressed in proportions or concentrations of total dry diet consumed daily. The maximum and minimum recommended intake of these elements can be safely tolerated by the body. It is observed that the availability of mineral elements is affected by the chemical form in which it is ingested. The gross dietary intake of an element does not necessarily reflect minimum or maximum requirement or tolerance [4]. A correlation of elemental concentration with its physiological effects can be seen in Fig I.1.

It may be emphasized that our knowledge of trace element distribution in the body, the delineation of deficiency situations in the organism, and the identification of trace element containing species depends on the contemporary state of the art in trace element analysis and as a whole to analytical techniques applicable to biological

systems. Several conferences and symposia have been organised to assess the current state of the art of trace element analysis (TEA) in medicine, biology.and nutrition.

I.3. INDIAN VEGETARIAN DIET

As already described there are several minor and trace elements which play vital role in human metabolism. All the essential elements are primarily supplied through diet which, however, may change depending on climate, regional geography, age, sex, health and socio-economic status [5]. Availability of an element to the body for biochemical processes depends on its absorption from diet which, in turn, depends on its chemical form (speciation) and composition of diet. Some elements though essential in trace amounts may turn out to be toxic at higher concentration. Thus it is important to determine the daily dietary intake [6]. Due to disparities between total dietary intake and its actual availability to the body, Food and Nutrition Board of the US National Academy of Sciences, have proposed the concept of

Recommended Dietary Allowance (RDS) which define the optimum daily dietary intake of nutrient elements [7].

(i) Dietary Survey Techniques : The conventional dietary survey techniques like diet, dietary interview and recording studies in combination with food tables are not suitable for the assessment of trace element intake. The food tables do not contain sufficient information on trace element contents. Therefore, it is not possible to account for addition or loss of trace elements during food processing. World Health Organization (WHO) has recommended three procedures for estimating the human dietary intake of trace elements :

(a) total diet collection of food reflecting a defined total diet of a consumer or a population,

(b) selective studies of individual foodstuffs, and

(c) duplicate portion sampling technique.

Much data are available in literature survey. During last few years large scale compilations and international programmes have been undertaken to monitor essential and

toxic elements in foods and diets from different parts of the world. In this regard efforts of WHO, Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA) have been noteworthy though many developed and developing countries have national level organisations responsible for such activities. There are innumerable studies from several countries to assess the dietary intake in respective population groups.

On the Indian scene, "Nutritive value of Indian Foods" was first compiled by Gopalan et. al., [1] under the aegis of National Institute of Nutrition (NIN) Hyderabad. This is being periodically revised and an updated version (in 1991) has been published by Narsingarao et. al. [1] Indian Council of Medical Research, New Delhi, have also published "Recommended Dietary Intakes for Indian" [7].

(ii) Habitual diets in India :

Extensive diet surveys carried out in different parts of our country both in rural and urban areas indicate that diets

are predominantly based on cereals. Diets of the poor continue to remain grossly inadequate for long time to come unless there is phenomenal improvement in their economic status to afford an adequate diet. The current production of milk, animal foods, vegetables, fruits, fats and oils are grossly inadequate to meet the needs of all the populations in the country according to the currently recommended nutritional standards [7]. Major thrust must be towards increasing the per capita availability of the protective foods.

I.4 ANALYTICAL TECHNIQUES

The fact that essential and toxic elements enter into organisms via the food chain together with the recommendations of the WHO, FAO and other bodies relating to daily allowances or maximum toierable intake of trace elements have necessitated the need for accurate and reliable determination of essential and toxic elements in various food articles. The composition of biological systems is so complex that the trace elements are totally masked by

major constituents. Some techniques, widely used in TEA are neutron activation analysis, atomic absorption spectromery flame emission spectrometry (AAS/AES)), X-ray fluorescence (XRF), spectrophotometery electroanalytical techniques and inductively coupled plasma-atomic emission spectrometry (ICP-AES), mass spectrometry (ICP-MS)

Each one of these techniques has its own advantages and limitations with regard to accuracy, precision, sensitivity, specificity, multielemental character, ease of handling, cost, automation etc. In following lines is given a brief account of these techniques.

(i) Neutron Activation Analysis : This method includes the bombardment of the sample with a beam of neutrons. Several types of neutrons of different energies are used to induce different kinds of nuclear reactions but thermal neutrons inducing (n, γ) reactions are most favourable. The γ-rays emitted after activation are detected using suitable

detection system. NAA can be of different types depending upon whether the chemical treatment is required or not. These include - Instrumental NAA (INAA), if no chemical treatment is done; Radiochemical NAA (RNAA), if chemical separation are carried out after irradiation to remove interferences.

Chemical NAA (CNAA), if chemical separations are carried out before irradiation and molecular activation analysis (MAA), if specific molecular components are determined [8]

The analytical procedure of NAA consists of two steps; irradiation of the sample with neutrons forming radioisotopes and then identification and measurement of induced radioactivity. The amount of radioactivity generated depends on the number of target atoms (N), the neutron flux, ϕ in $ncm^{-1}s^{-1}$, the neutron absorption cross section of the target nuclide, (σ) in cm^2 and the time of

irradiation (t) in s. Thus, the rate of formation of radionuclide is

$$\frac{dN}{dt} = N\sigma\phi \qquad \ldots \quad (I.1)$$

However, the product radionuclide starts decaying as soon as it is formed, so that the net production rate can be represented as :

$$\frac{dN^*}{dt} = N\sigma\phi - \lambda N^* \qquad \ldots \quad (I.2)$$

where λ is the decay constant. After integrating eqn. (I.2) and rearranging, we get

$$\text{Activity (A)} = N\sigma\phi(1-e^{-\lambda t})e^{-\lambda d}.\varepsilon \qquad \ldots \quad (I.3)$$

where d is the delay time and ε is the efficiency of detection system .

For a set of experimental conditions, σ and ϕ can be assumed to be constant so that $((1-e^{-\lambda t})$ is the limiting factor also called saturation factor for the maximum growth of

activity in a sample. Number of target atoms (N) is defined as

$$N = \frac{w \times i \times 6.023 \times 10^{23}}{M}$$

where i is the isotopic abundance of the target nuclide and M its atomic mass. On substitution

$$A = \frac{w.i.\sigma.\phi}{M}(1-e^{-\lambda t})e^{-\lambda d}.\varepsilon.6.02x10^{23} \quad \ldots \quad (1.4)$$

The eqn (I.4) gives theoretical estimate of the activity generated in a sample. However, due to uncertainties in the determination of σ, ϕ and absolute activity of product radionuclide, comparator or standard with known concentration is always irradiated along with the unknown sample under the same experimental conditions. Since activity of a radionuclide is proportional to the elemental content, the ratio of activities for the sample and standard will be :

$$\frac{A_{samp}}{A_{std}} = \frac{W_{samp.}}{W_{std.}} \quad \text{or,} \quad W_{samp} = W_{std} \cdot \frac{A_{samp}}{A_{std}} \quad \ldots \quad (1.5)$$

If amount of the sample and standard are different, the activity can be rationalised to specific activity (or count rate, R = Activity $g^{-1}s^{-1}$). Thus, $w_{samp} = w_{std} \times \frac{R_{samp}}{R_{std}}$ ... (I.6)

Therefore,

concn. of element in sample = Conc. of the element in std. $\times \frac{\text{sp. activity of samp}}{\text{Sp. activityin of std.}}$

This equation can be directly used for the calculation of concentration of an element in an unknown sample provided its concentration in the standard is known.

Flame photometry and flame emission spectrometry is a special area of emission spectrometry in which a flame is used to excite the atoms. In this technique, excitation is brought about by spraying a solution of the sample into a hot flame. The basic principle of flame emission spectroscopy rests on the fact that salts of metals when introduced under carefully controlled conditions into a suitable flame, are vapourised and excited to emit radiations that are characteristcs of each element.

(ii) Atomic Absorption Spectrometry : This technique involves the study of the absorption of radiation (usually in the ultraviolet - visible region) by neutral atoms in gaseous state. The sample is first converted into atomic vapours and then the absorption of atomic vapour is measured at a selected wavelength which is characteristic of each individual element. The measured absorbance is proportional to the concentration and analyses are made characteristic of each individual element. The measured absorbance is proportional to the concentration and analyses are made by comparing this absorbance with that of the standard solution of known composition under the same experimental conditions.

(iii) X-Ray Fluorescence : The fluorescent emission by γ-rays provides one of the most potent tools available to the analyst for the identification and measurement of heavy elements in the presence of each other and in nearly any matrix. In this technique the sample is irradiated by a

primary beam of x-rays, which have the same effect as a beam of energetic electron, i.e., the ejection of electrons from inner orbital shells. Each heavy element in the sample is caused to emit radiation of the same frequencies it would if it were made the target of a separate X-ray tube. The radiation from the sample is analysed with the aid of a goniometer and detector.

(iv) Spectrophotometry : In this technique the sample is irradiated with the electro-magnetic radiations of a particular wavelength and of defined energy. Then the amount of light absorbed emitted at a particular wavelength and energy absorbed or emitted by the sample is recorded and thus concentration is calculated using Beer-Lambert law..

(v) Electroanalytical Techniques : These techniques are based on electrochemical behaviour of the system. Three electrical properties - current (i), resistance (r) and voltage (v) are measured as a function of time to carry out the

analysis. Electrogravimetry, conductometry, coulometry, potentiometry and voltammetry all come under this heading.

Voltammetry deals with the current - potential relationship in an electrochemical cell or with the current-time response of an electrode at a controlled potential. Polarography is the name applied to dc voltammetry at the dropping mercury electrode. It has been most extensively used technique for elemental analysis in a variety of matrices. The electrolyte is a dilute solution of the material under examination (which must be electroactive) in a suitable medium containing an excess of an indifferent electrolyte (base or ground solution, or supporting electrolyte) to carry the bulk of the current and raise the conductivity, of the solution thus ensuring that the material to be determined, if charged, does not migrate to the dropping mercury cathode. From an examination of the current voltage curve, information as to the nature and concentration of the material may be obtained.

(vi) Inductively Coupled Plasma (ICP) Methods : When energy is imparted to a sample via sufficiently high temperature environment, the elements in the sample are converted to excited free atoms and ions. As they return to the ground state, they emit radiation at characteristic wavelength from which their concentration may be determined.

The ICP technique is used for elemental analysis of liquid samples. The sample is passed through a nebuliser into a plasma which exists in and is maintained by a high frequency electromagnetic field. It is called as inductively coupled plasma-atomic emission spectrometry (ICP-AES). Another modification of ICP technique is inductively coupled plasma-mass spectrometry. ICP-MS where molecular mass of the compound and its elemental composition can be determined most accurately. In this technique, molecules are bombarded with a beam of

energetic electrons. The molecules are ionised and broken up into many fragments, some of which are positive ions. Each kind of ion has a particular ratio of mass to charge, i.e. m/e ratio. Thus the metal ion concentration could be determined.

The advent of ICP-AES, ICP-AFS and ICP-MS, all with multielement capabilities, has slowed the AAS/AES market. However, AAS/ARS technology is deeply entrenched in the field of analytical chemistry and it will be years before it is replaced by the methods listed above or other new technologies..

I.5 ATOMIC ABSORPTION SPECTROMETRY (AAS)

In this technique sample in the form of solution is aspirated into the flame to convert the element into the atomic vapor state. Most of the atoms in flame remain in the ground state which are measured in atomic absorption. The amount of light from a source that is absorbed by the atoms

is measured. The absorption follows Beer's Law as the absorbance is directly proportional to the path length in the flame and to the concentration of atomic vapor in the flame. A calibration curve of concentration in the solution versus absorbance is plotted and the concentration of the unknown is calculated. An atomic absorption spectrophotometer consists of a light source, a cell (the flame), a monochromator, and a detector. The flame is placed between the source and the monochromator. The source used is almost exclusively a hollow cathode lamp, which is a sharp line source, characteristic of the element to be determined..

Two types of burners are used for atomisation total consumption burner and premix type. The most widely used flame for atomic absorption is the air-acetylene flame with a premix burner. A monochromator is used to isolate the resonance line from all non absorbed lines emitted by the radiation source. Photomultiplier is used for detecting the signal and these are displayed on the chart recorders.

I.6 QUALITY CONTROL (QC)

It comprises of a set of experimental and statistical procedures designed to test systematically and continually, whether a measurement process is in a state of statistical control and capable of producing data with confidence. Only the measurement data, obtained in conjunction with a good quality assessment can meet the criterion of transparent and self-evident reliability. Several standards known as Standard/Certified Reference Materials are analysed along with the sample and the results are compared with the certified values. These standards are obtained from various international agencies such as National Institute of Standards and Technology (NIST) USA, International Atomic Energy Agency (IAEA) Vienna, National Institute of Environmental Studies (NIES) Japan etc. If the results obtained for the SRMs agree with the known certified concentration within the expected uncertainty, the corresponding results for the unknown sample acquire

considerable, if not absolute, confidence. The quality of analytical data also depends on the minimisation of errors at each stage. For data of high accuracy and precision quality assurance (QA) is absolutely essential [12]. Quality Assurance (QA) derives from all measures taken to ensure that laboratory results are reliable, including the adoption of scientifically and technically sound practices, the selection, collection, and transport of specimens; and the recording, reporting and interpretation of results. It also implies training and management of the personnel to improve the reliability of investigations [13]. Quality control (QC) as a part of QA is aimed at verifying that analytical results, issued from the laboratory, meet specific requirements or the needs of the user. The most effective way to carry out data QA controls is to analyze, using the same procedure, a standard material with well-known and established data on the trace element under investigation.

In view of economic liberalisation and globalisation it has become all the more essential for our R&D laboratories to stress upon the need for quality assurance. There are several approaches adopted for this purpose e.g., multiple analysis at regular intervals, analysis of the sample by more than one techniques and then perform statistical analysis for testing significance of data. Another method for testing reliability is analyzing Standard Reference Materials (SRMs) after a set of interval [14, 15].

I.7 STANDARD REFERENCE MATERIALS (SRMs)

Standard or Certified Reference Materials are one of the keys to the achievement of reliable and accurate results which are the basis for the proper monitoring of the environmental and biological systems and consequently for correct decisions to be taken to maintain or improve the situation. The use of the SRMs in laboratories ensures the possibility of providing accurate results which are also traceable to recognized international reference standards and

which can be compared with the results for many other laboratories. An unique monograph *"Biological Reference Materials Availability, Uses and Need for Validation of Nutrient Measurement"* edited by W.R. Wolf deals with various topics on the preparation of CRMs quality control materials and specimen banking (16) by illuminaries from all over the world.

A large number of very reliable geological, biological and environmental Reference Materials are available from well established national and international bodies such as US Geological Survey, NIST (formerly NBS) and the BCR (Community Bureau of Reference). In these institutions SRMs preparation (i.e., both homogeneity testing and certification of analyte contents) is based on analytical data obtained in their own laboratories using a definite or absolute method, two or more references or highly reliable methods, and/or on data obtained in a qualified network of experienced labs using the above mentioned methods.

The National Institute for Environmental Studies (NIES), Japan have also issued a variety of biological and environmental reference materials to serve the needs of scientific community engaged in elemental analysis of such matrix materials.

International Atomic Energy Agency (IAEA), Vienna is one of the prime organizations actively involved in the development of Certified Reference Materials (CRM) of biological, geological, environmental and other matrices. These SRMs are distributed to various laboratories around the world through Analytical Quality Control Services (AQCS) at their Seibersdorf Laboratories.

Biological SRMs are of much recent origin compared to environmental and geological standards which have been available for a long time. The first major preparation and distribution of a biological RM was accomplished by Prof. H.J.M. Bowen of Reading University, UK in 1964. Bowen's

Kale has been most widely analysed employing a host of analytical techniques and its "most likely" values for various elements have been periodically compiled [17]. IAEA Vienna a have taken a leading role in issuing biological SRMs over the last several years. Because of large amount of time and effort involved, only a small number of elements have been certified to date in a small number of biological matrices.

Before developing a reference material one of the first considerations should be to assess the technical requirements which dictates its end use. This governs the certification requirements such as accuracy, stability and physical form of the material. It is also necessary to develop adequate means of measuring the property and preferably one which is traceable to the appropriate SI units. Some of the essential requirements of a reference material are [18]

- Similarity with sample matrix,

- Stability and definite composition over a long period,
- Homogeneity with regard to sample size and phase segregation,
- Minimum moisture content or preferably in dried form,
- Radiation sterilized (specially for biological samples),
- Methodology followed for element certification.

On the Indian scene, some individual efforts have been made at BARC and other places. Recently a cell has been started at the National Physical Laboratory, New Delhi for the development of suitable SRMs for environmental analysis.

I.8 LITERATURE SURVEY

During last 2-3 decades large scale compilations and international programmes have been undertaken to monitor essential and toxic trace elements in foods and dietary components. In this regard efforts of WHO, FAO and IAEA have been noteworthy though many developed and developing countries have their own national level

organizations responsible for such activities. Amongst earliest workers Underwood [4] first dealt with the subject matter in his famous monograph, Trace Elements in Human Nutrition published in 1971 but later revised. Another monograph Crossby [19] reviewed the determination of metals in foods using a variety of analytical techniques. It deals with nutritional in the field was written by A.S. Prasad in 19 but recently its updated version *Essential and Toxic Trace Elements in Human Health and Disease* has been published in 1991 biochemical and toxicological aspects of trace elements citing 346 references.

There are innumerable studies from several countries to assess the dietary intake in respective population groups. On the Indian scene, "*Nutritions Value of Indian Foods* was first compiled by Gopalan et. al. [1] under the aegis of National Institute of Nutrition (NIN), Hyderabad. This has been periodically revised and recently an updated version has been published by Narsingarao et. al. [1]. Several

different techniques have been employed for the analysis of biological materials, most common being AAS and NAA though ICP-AAS has also been employed quite frequently.

The analysis of foods and related materials such as plant and animal tissues for a wide range of less common elements has been reported in literature, most common technique being atomic absorption spectrophotometry AAS. Welch and Cary [20] measured concentrations of chromium, nickel and vanadium in plant materials.

Cervera et al. [21] determined As in tomato products by dry-ashing hydride generation AAS. Sakae et al. [22] have reported analysis of Mn, Fe, Zn and Cu in plant materials by alkali fusion flame AAS. Puchades et al [23] have followed rapid digestion procedure for the determination of Pb in vegetables by electrothermal atomization AAS. Miller-Ihli [24] has reported simultaneous determination of 16 elements in biological materials using a multielement AAS system (SIMAAC). Shen et al [25] deter-

mined Cr in vegetable suspensions using flameless AAS. Lynch and Littlejohn [26] developed a slurry atomization method for the determination of Cd in food samples by electrothermal AAS. Xu et al [27] reported a rapid method for the determination of Cu in vegetables by AAS. Barbera et al [28] determined Mn in vegetables by AAS. Poluyanov and Zeinaloo [29] determined Mo in plants by extraction of the picrate with $CHCl_3$ and then following AAS technique. The method has been found sensitive, selective and interference free. Butcher et al [30] analysed food and agricultural standard reference materials for Tl, Mn and Pb by electrothermal-atomizer spectrometry. Jin and Cheung [31] determined Co, Ni, Cu, Zn, As, Mo and Sr in cabbage, turnip, soyabeans and soil by inductively coupled plasma mass spectrometry (ICP-MS).

Jackson and Alloway [32] determined toxic element Cd in plant tissues by electrothermal atomization AAS employing matrix-analyte modification and Smith-Hieftje

background correction. Alvarez and Capar [33] determined Se and As in foods by continuous hydride generation AAS method. Contents of toxic heavy elements such as Cd and Pb have been reported by Barbera et al. [34] using flame AAS. Miller-Ihli and Green [35] have reported determination of Cr in foods and biological materials which were first dry ashed and then analysed by graphite-furnace AAS. Peak areas were directly calibrated against aqueous standards. Sun et al [36] analysed peach leaves and tomatoes for 15 elements in aqueous solution containing HNO_3 by ICPAES after digesting with HNO_3 - HF - $HClO_4$ mixture.

Ohta et al. [37] have analysed biological materials such as bovine liver, milk powder and Kale for Mn using electrothermal atomization AAS.

Schuhmacher et al. [38] determined Co, Cu, and Zn concentrations in some Spanish vegetables such as radish root, celery, potato, onion, leek, chard, spinach, lettuce, endine, cauliflower, cabbage, tomato, green pepper,

artichoke, green bean and eggplant by employing AAS and ICP-AAS. In majority of analyses reported in literature, emphasis has been on the development of suitable methods for dissolution of plant material and then an element or few have been determined using suistable modifiers and interference free values were obtained. Also reported are recovery, sensitivity and other parameters.

Takacs et al [39] analysed tomato plants for 13 to 20 trace elements by PIXE and ICP techniques. Sodium and potassium are primarily electrolytic elements but their contents are important in plant materials. K contents in biological material were determined by Ohta et al [40] by electrothermal AAS using a Mo tube atomiser. Similarly, Lima et al. [41] have determined K and Na in vegetables by AES using a flow injection system with two dialysis units. Mingorance et al [42] reported B, Cd, Cu, Fe, Mn, P, Pb and Zn in some biological samples by AAS.

Vinas et al [43] reported Ca, Mg, Fe, Zn and Mn in cauliflower, bean, citrus and apple leaves by flow injection AAS using standard addition method down to detection limits of few μg/g. Wang and Zuo [44] determined K, Na, Ca, Mg, Mn, Cu, Zn and Fe in nutrient foods by flame-AAS.

Besides AAS, another most widely used technique has been NAA(instrumental as well as radiochemical) for multielemental analysis of biological plant leaves. Dijingova et al [45] analysed several reference materials for various trace elements by INAA and flame AAS. Results obtained by two methods have been compared. Kucera and Byrne [46] have analysed biological materials by fast-neutron radiochemical activation analysis. The samples were first dry-ashed and then irradiated for 85 hr. Results agreed well with certified and literature values. Becker et al. [47] have determined 30 elements using instrumental NAA, prompt gamma-ray neutron activation analysis (PG-NAA) and radiochemical NAA for certification of tomato leaves,

SRM-1573a. Becker [48] also reported 21 elements by INAA for certification of SRM 1570a, spinach. Carmo Freitas [49] reported elemental concentrations in the spinach reference material by k_0 based INAA. Omote et al. [50] analysed Ca,Mg,K,P,Al and Fe by X-ray fluorescence. Another spinach sample was developed as CRM by IAEA and its analysis for 40 elements by INAA has been by Ni et al [51]. Naihara et al [52] carried out radiochemical separation for the certification of some trace elements in biological reference material by NAA. It is reported in literature that INAA is the most favoured technique for certification of reference materials at NIST (USA) and IAEA (Vienna). It is primarily because of its higher senstivity and multilelemental characters.

Samudralwar et al. [53] carried out multielemental NAA of an IAEA intercomparison standard Hay Powder, V-10 and some edible plant leaves consumed in India. A novel method of NAA was developed for the determination of P in

biological materials of plants and tissues. The method inovles reactor irradiation for 6-8h followed by counting of β particles ^{32}P after 2-3 weaks cooling of sample.

Saraswati and Watters [54] have reported As and Se in reference materials (spinach and tomato leaves) using flow injection AAS. Wu et al. [55] determined 18 mineral elements in vegetables by ICP-AES. The method was applied to asparagus, common perilla, garlic and radish. Qin et al. [56] carried out direct determination of B in botanical samples by fluorination and electrothermal vaporization ICP-AES. Yaman and Gucer [57] reported Cd and Pb in vegetables after activated carbon enrichment by AAS. Anderson [58] analysed 17 elements (Na, K, Mg, Ca, V, Ca, Mn, Fe, Ce, Ni, Cu, Zn, Mo, Cd, Pb, P and S) by micro digestion followed by ICP-AES.

Miller-Ihli [59] carried out analysis of Ca, Cu, Fe, Mg, Mn and Zn in 32 fruits by AAS and K and Mn by AES after wet ashing. Hoenig et al. [60] developed a wet digestion

method for the mineralization of plant samples. It includes dissolution in HNO_3 and H_2O_2 as well as HF treatment followed by AAS. Recently Wieteska et al. [61] developed extraction as a method for the preparation of vegetable samples for the determination of eight elements (Al, Ca, Cd, Cu, Fe, Mg,) by AAS.

I.9 AIM AND SCOPE OF THE PRESENT WORK

Life cannot be sustained without adequate nourishment. Man needs adequate food for growth, development and to lead an active and healthy life. Plants can manufacture the food they need from simple chemicals derived from the soil, water and carbondioxide of the air in presence of sunlight. However, human beings procure the elements from their diet as their direct intake is not possible, if taken they are not easily absorbed by the system. The food contains the elements in bioavailable form, so they can be easily assimilated by the system. In several cases elemental

deficiencies leading to diseases or chronic toxicity due to heavy elements have been reported.

The foods contain many elements that are nutritionally important like Ca, Mg, P, Na, K, Fe, Cu, Zn, Cr, F etc. and it also contains some elements which are environmental contaminants such as Cd, Hg, As, Br, Pb. The human body is composed of many major, minor and trace elements which are essential for various life processes. The concentrations of these elements are to be within certain limits so is the case with vegetables. As already evidenced in preceeding section, several workers all around the world have analyzed vegetable plant leaves for their elemental contents. However, only scanty efforts have been made to analyses Indian vegetables and plant leaves.

In this project we have attempted to analyses several green vegetables consumed as part of our daily diet for some essential elements and environmental contaminants

from industrial effluents. Vegetable samples were procured from the maket, dried at < 90° C and powdered. It was then made into solution and analysed for 11. essential elements including some environmental pollutants by AAS. Five biological Standard Reference Materials including Bowen's Kale, tomato leaves (SRM-1573), citrus leaves (SRM-1572), Cabbage (CRM-359) and spinach (CRM-331) procured from National Institute of Standards and Technology, USA and International Atomic Energy Agency, Vienna were also analysed for quality control and data validation. Also for some samples, moisture content and ash contents were deterimined. Spinach and mint samples were also analysed by NAA and iron was further determined spectro-hotometrically. Replicate analysis were performed for precision measurement.

From these studies elemental intake may be calculated and one could learn about environmental contaminants including their source of origin. From the relative elemental

contents, enrichment of various vegetables may be estimated and in case of deficiency suitability of particular vegetables may be predicted for population groups.

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

II.1 REAGENTS AND CHEMICALS

All the reagents and chemicals used are of the following specifications :

(i) 1,10 Phenanthroline (monohydrate), $C_{12}H_8N_2.H_2O$, G.R., E. Merck.

(ii) Hydroxyl ammonium Chloride, $NH_2OH.HCl$, L.R., S.D. Fine Chemicals

(iii) Sodium acetate, $CH_3COONa.3H_2O$, A.R., E. Merck.

(iv) Ferrous Ammonium Sulphate, $(NH_4)_2SO_4.FeSO_4.6H_2O$, A.R., Glaxo Laboratories

(v) Cadmium Sulphate, $CdSO_4$, A.R., Central Drug House

(vi) Magnesium Sulphate, $MgSO_4.7H_2O$, G.R., Loba-Chemie

(vii) Calcium Sulphate, $CaSO_4.2H_2O$, L.R., Glaxo Laboratories

(viii) Potassium Dichromate, $K_2Cr_2O_7$, A.R., Qualigens

(ix) Copper Sulphate, $CuSO_4.5H_2O$, A.R., S. Merck.

(x) Lead Nitrate, $Pb(NO_3)_2$, A.R., BDH

(xi) Zinc Sulphate, $ZnSO_4.7H_2O$, A.R., Qualigens

(xii) Manganese Sulphate, $MnSO_4$, A.R., BDH

(xiii) Nickel Chloride, $NiCl_2.6H_2O$, A.R., Merck

(xiv) Cobalt Chloride, $CoCl_2.6H_2O$, A.R., BDH

All the acids used were of AR grade. HCl, HNO_3, H_2SO_4, $HClO_4$, were of Qualigens and CH_3COOH was of Merck.

II.2 SAMPLING

All vegetables were procured from the local market. The leafy vegetables and others were cleaned visually to sort out only soft and healthy leaves. The hard sticks or stems were removed. The sorted leaves were then washed thoroughly with distilled water to remove all the dust and mud These were further washed with distilled water and then after swiping with tissue paper., weighed on a beam balance. The plant material was first dried in an oven to evaporate the water and then dried under IR lamp at temperature $< 80^{\circ}C$. The dried plant material was grounded to a fine powder in an agate mortar. This was again weighed to calculate the moisture content of the vegetable sample. Their local names including that in Hindi and botanical names are given in Table II.1. In addition to 14 green vegetables three varieties of cauliflower were procured from the Botany Department, Nagpur University, Nagpur. These were cauliflower, broccoli and their hybrid.

TABLE II.1 LIST OF NAMES OF VEGETABLES IN ENGLISH/HINDI AND THEIR MOISTURE & ASH CONTENTS

S.No.	English(Hindi)	Botanical Name	Moisture (%)	Ash (%)
(i)	Spinach (Palak)	*Spinacea oleracea*	93.6	21.1
(ii)	Fenugreek (Methi)	*Trigonella foenum graecum*	ND	ND
(iii)	Cabbage (Patta Gobhi)	*Brassica oleracea*	93.8	8.4
(iv)	Mustard (Sarson)	*Brassica campestris var. sarson*	ND	ND
(v)	Bathua (Bathua)	*Chenopodium album*	ND	ND
(vi)	Gram leaves (Chana Saag)	*Cicer arietinum*	ND	ND
(vii)	Neem (bitter)	*Azadirachta indica*	76.9	6.8
(viii)	Curry leaves (Neem Sweet)	*Murrtaya koenigii*	78.6	12.0
(ix)	Mint leaves (Pudina)	*Mentha spicata*	86.6	8.8
(x)	Radish leaves (Mooli patta)	*Raphanus sativus*	87.2	13
(xi)	Coriander leaves (Dhania patti)	*Coriandrum sativum*	87.5	9.5
(xii)	Capsicum (Shimla Mirch)	*Capsicum annum*	92.3	6.64
(xiii)	Beans (Sem)	*Phaseolus coccineus*	90.7	6.5
(xiv)	Singhri (Seng)	*Pithacellobium dulce*	90.4	7.1
(xv)	Cauliflower (Phool gobhi)	*Brassica oleracea var. botrytis*	91.7	ND
(xvi)	Broccoli	--	89.9	ND
(xvii)	Hybrid	--	90.2	ND

After drying, these were powdered in agate mortar and sieved through 100 mesh to keep the particle size uniform and homogeneous. The resultant fine powder was stored in plastic containers. Following Standard Reference Materials (SRMs) of botanical origin were also analyzed along with the samples.

(i) Kale from Prof. H.J.M. Bowen [62] University of Reeding, U.K.

(ii) Citrus leaves (SRM - 1572), from National Institute of Standards and Technology, USA [63].

(iii) Tomato leaves (SRM-1573), from National Institute of Standard and Technology, USA (64).

(iv) Spinach (CRM-331), from International Atomic Energy Agency, Vienna, (65).

(v) Cabbage (CRM-359), from International Atomic Energy Agency, Vienna, (66).

These were used as such without any further treatment.

II.3 EQUIPMENTS

All the samples were analyzed on Perkin-Elmer 3100 Atomic Absorption Spectrophotometer, USA. It is designed for the use with flame absorption, flame emission and mercury/hydride atomic absorption techniques. It has a high efficiency burner system which offers both a flow spoiler and impact bead for optimal performance with all sample types.

Various parameters such as lamp current, integration time, number of replicates, calibration type, AA-technique are fed into the software and then the instrument's performance is optimised. A hollow cathode lamp is installed and wavelength is set corresponding to the element to be determined. The wavelengths of measurements for individual elements and their sensitivity are given in Table II.2. The burner head locking ring is turned clockwise until it is properly seated. Acetylene-air mixture gas is turned on and the burner height is adjusted to ignite the flame. A sensitivity check standard is used for calibrating the instrument and then other standards are run which have their concentrations less than the sensitivity check.

Table II.2 : Wavelengths And Detection Limits Of The Elements Analysed By AAS

ELEMENT	WAVELENGTH (nm)	SENSITIVITY (μg/g)
Ca	422.7	4.0
Cd	228/8	1.5
Co	240.7	7.0
Cr	357.9	4.0
Cu	324.8	4.0
Fe	248.3	5.0
Mg	285.2	0.3
Mn	279.5	2.5
Ni	232.0	7.0
Pb	283.3	20.0
Zn	213.9	1.0

An UV - visible spectrophotometer, UV-1601, Shimadzu was used for the spectrophotometric determination of Fe by o-phe nanthroline complex method spectrophotometrically. The absorbances recorded down from both the techniques were used to calculate the concentrations of the elements by the linear regression method.

II.4 SAMPLE DISSOLUTION

The inorganic content of most foods is only a minor constituent and is interfered by major constituents. Therefore, organic matter is removed before the final determination of the element of interest. As there have been many methods for the determination of elements, there are also various methods proposed for the destruction of organic matter. Individual workers have their own prefrences. The early work has been reviewed critically by Middleton and Stuckey [67]. A more comprehensive evaluation of the many alternative methods is described by Gorsuch [68], who used radiochemical technique to investigate losses of many elements using a wide range of mineralisation conditions in experiments.

Despite a large number of methods that have been proposed, organic matter is usually destroyed by oxidation, either by the use of oxidising acids or in the dry state with atmospheric oxygen. Any method used represents a compromise between conflicting objective, viz. complete recovery of the element to be determined, speed, convenience, requirement of the end-determination technique and safety. Another way is wet oxidation by acids and has the advantage of being applicable to a wide variety of samples, fairly rapid and less prone to volatilisation or retention losses. The oxidation reagents commonly employed include nitric, sulphuric and perchloric acids and hydrogen peroxide. As each reagent possesses inherent advantages, recommended method frequently use one or combination of these reagents. For the analysis of food items combination of the above reagents are normally recommended.

Nitric and sulphuric acid mixtures are widely used for the decomposition of plant material, while mixtures of nitric, sulphuric and perchloric acid are used for materials containing fats, which decompose only with difficulty. Nitric acid - perchloric acid mixtures has been recommended for the destruction of protein and carbohydrate, but not

when fat is present. Wieteska et al. [61] have suggested an alternative simple and rapid technique for the quantitative isolation of Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn from vegetable materials. It involves the use of a mixture of conc. HCl, dil. HNO_3 and HF acids. It allows to obviate the operation of destruction of an organic matrix and at the same time to dissolve the elements quantitatively.

Recently, Hoenig et. al. [60] have developed a wet digestion method for the mineralisation of plant samples. Besides the use of HNO_3 and H_2O_2 it also includes HF treatment followed by an evaporation to dryness so that all the elements may be retained along with insoluble silica residue. This wet digestion method may be applied to plant sample using both classical heating devices (sand-bath) or microwave oven system. We have followed the following procedure, using a combination of HCl, HNO_3 and $HClO_4$.

About 1g of the dried vegetable sample was weighed accurately and taken in a beaker to which 15 ml 5MHCl was added and then heated on the hot plate. After sometime a slurry is formed which is cooled and then 10 ml conc. HNO_3 was added dropwise. The mixture was continuously stirred till all the fumes vanished. It was further heated till a

clear solution was obtained. On further heating if white gelatinous mass was observed, 50 ml of distilled water was added. It was then heated to reduce the solution up to 10 ml and then reducing further to a slurry few drops of aqua-regia were added and heated. Added water to see if all the organic matter was oxidised, if not then 2 ml of perchloric acid was added. After that 5 ml of conc. HCl was added and the solution was filtered through Whatman filter paper No. 42 to remove turbidity. The solution was made up to 50 ml. All the solutions were stored in tightly capped polythene bottles. These were directly used for various trace element determination using AAS.

II.5 MOISTURE AND ASH CONTENT

The ash content of ten samples was determined by taking approximately 0.5 g of the sample in a silica crucible and then these were heated at 500°C for 3 hours in a muffle furnace. Firstly, the silica crucibles were heated and cooled to obtain a constant weight.

The fresh vegetables were weighed and dried and again weighed. The difference in weight gave the moisture content and then the percentage was calculated. Ash content and percentage moisture content are listed in Table II.1.

II.6 SPECTROPHOTOMETRIC DETERMINATION OF IRON

Iron was also determined spectrophotometrically using o-phenanthroline. In this method, Fe^{2+} reacts with o-phenanthroline to form an orange-red complex $[(C_{12}H_8N_2)_3Fe]^{2+}$. The colour inensity is independent of acidity in the pH range 2-9 and is stable for long period. In case of Fe^{3+}, it is reduced with hydroxylammonium chloride or with hydroquinone.

Ferrous iron forms a reddish-orange complex absorbing at 515 nm whereas in case of and ferric mixture a yellow ferric complex with identical absorption at 396 nm where the amount being additive, is formed. The solution, slightly acidic with H_2SO_4 is treated with o-phenanthroline and buffered with potassium biphthalate at a pH of 3.9. Absorbance at 396 nm gives the total iron and that at 515 nm only ferrous iron is measured. Following solutions were prepared :

(i) o-phenanthroline; (0.25% solution in water) 0.25 g of 1,10-phenanthroline monohydrate was dissolved in 100 ml distilled water.

(ii) Acetate Buffer : 65 ml of 0.1M acetic acid and 35 ml of 0.1M sodium acetate were mixed to obtain the buffer of the pH, 4.5.

(iii) Hydroxyl ammonium chloride, 10% solution was prepared by dissolving 10 g $NH_2OH.HCl$, in 100 ml of water.

(iv) 0.1M Iron solution : 3.9216 g of FAS was dissolved in 100 ml of distilled water. Few drops of H_2SO_4 were added before making up. As the detection limit of this method is between 0.5-5 ppm, it was diluted to 5 ppm.

Procedure : Five standards were prepared by taking 5, 10, 15, 20 and 25 ml each of 5 ppm iron solution in 50 ml volumetric flasks. In each case 5ml of hydroxyl ammonium chloride was added to reduce Fe^{3+}, if any. To maintain the pH between 3-6, 5 ml of acetate buffer was added and then 4 ml o-phenanthroline was added. It gave a red coloured complex whose volume was made up to 50 ml. The same procedure was repeated for the unknown solutions of vegetables where iron is to be determined. The solutions were kept for sometime and then absorbances were measured. Absorption spectrum and calibration plot are shown in Fig. II.1.

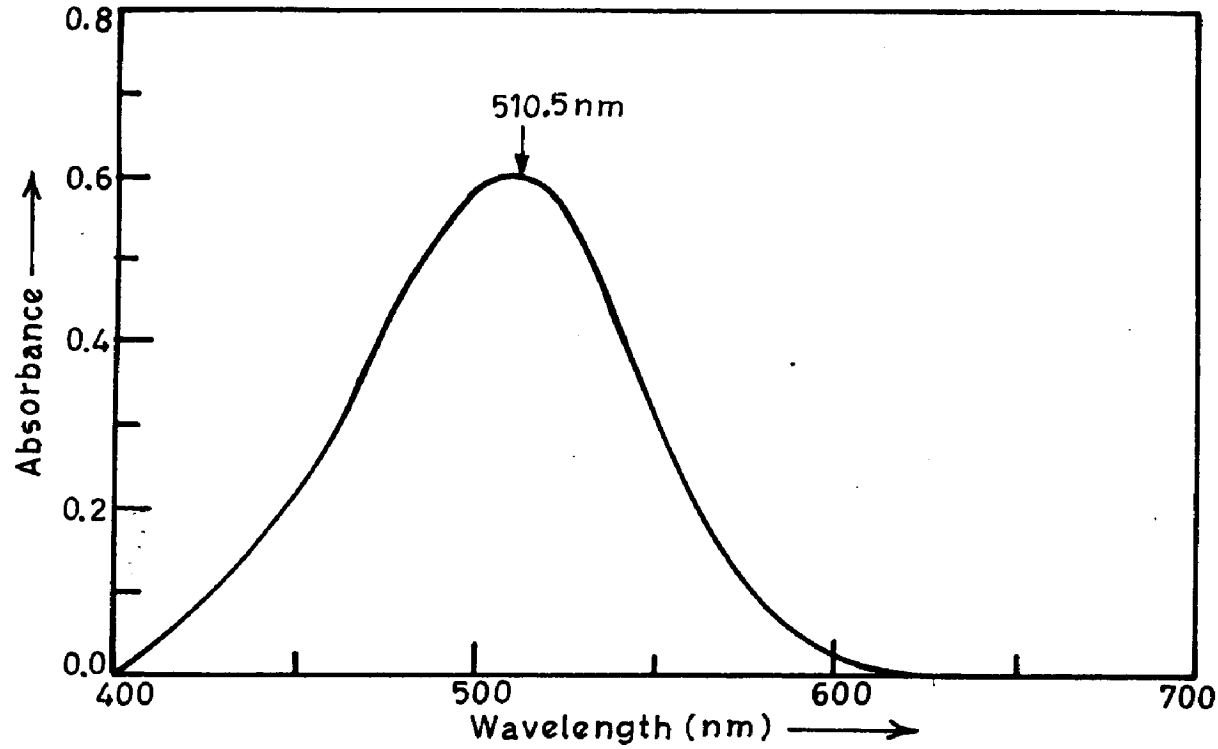

Fig II.1(A). Absorption spectrum of iron(II)-phenanthroline complex.

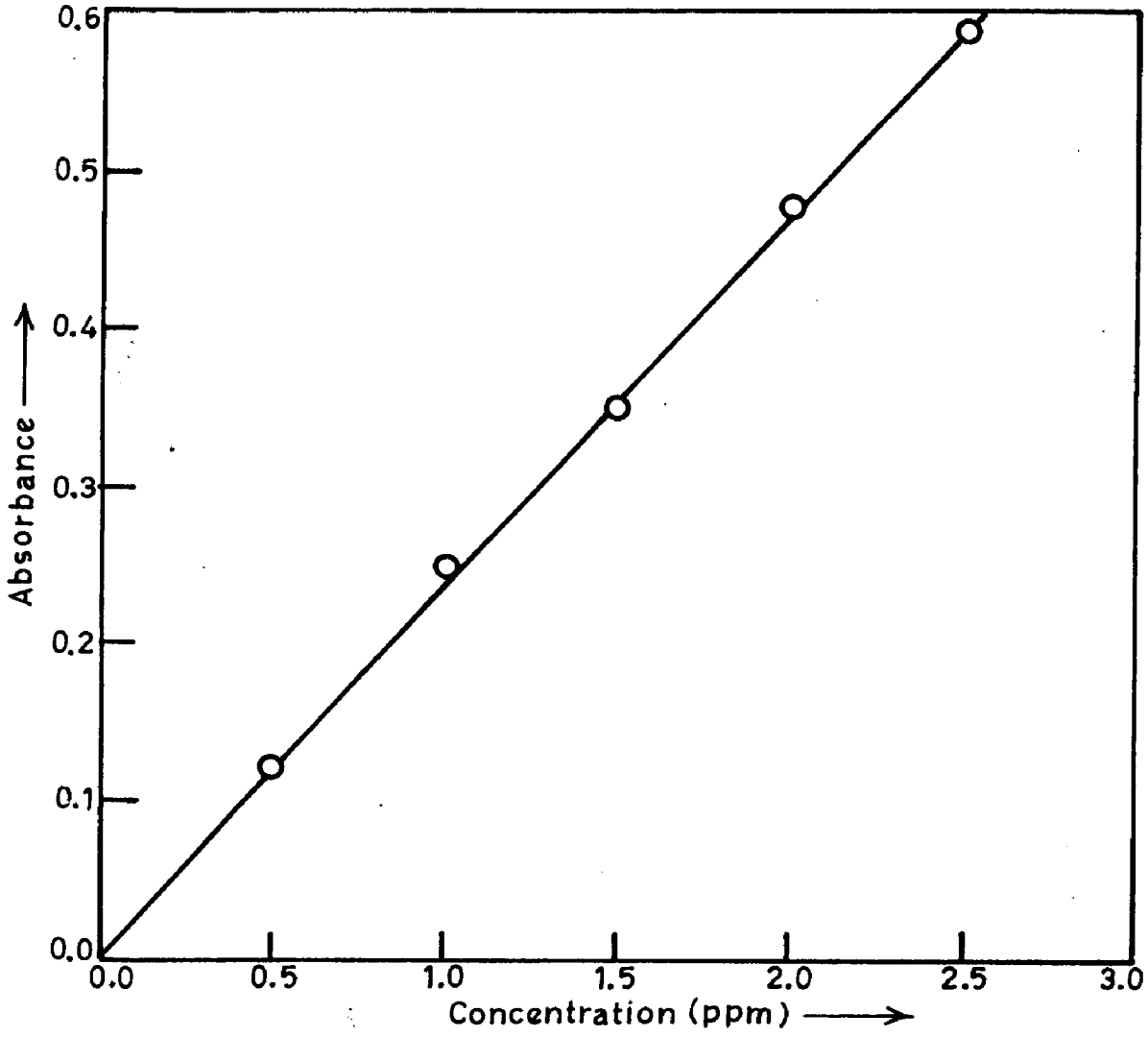

Fig.II.1(B). Calibration plot for the spectrophotometric determination of iron.

II.7 MULTIELEMENTAL ANALYSIS BY NAA AND PGA METHODS

Two samples of spinach and mint and one standard reference material Bowen's Kale were analysed by NAA and PGA for their various elemental concentrations.

100 mg sample each of spinach and mint along with Bowen's Kale as comparator standard were irradiated with thermal neutrons at ~ 10^{12}n $cm^{-2}s^{-1}$ in Rikkyo University Reactor (TRIGA Mark-II) Japan for 6 hours. After suitable cooling samples were counted on an HPGe (EG and G ORTEC) detector coupled with 4k MCA at successive intervals of 3d, 5d, 8d and 11d at the Radioisotope Research Centre of Tokyo Metropolitan University. Typical gamma ray spectra are shown in Fig. II.2, 3 & 4, where characteristic gamma photopeaks corresponding to individual radionuclides are identified. The peak areas were calculated using the software and elemental concentrations were calculated using the eqn.

$$\text{Concn. of Element in Sample} = \text{Concn. of Element inStd.} \times \frac{\text{Sp. Activity in Sample}}{\text{Sp. Activity in Std.}}$$

For PGA, samples were irradiated with a pulse cold neutron beam at Japan Atomic Energy Research Institute and the characteristic γ-rays

emitted were measured wherefrom elemental concentrations of B, Ca, Mg, Na, K, Cl, H and S were calculated.

RESULTS AND DISCUSSION

CHAPTER III

RESULTS AND DISCUSSION

III.1 RESULTS

Our data for elemental concentrations in the Biological Standard Reference Material are given in Table III.1. Also included in the table are certified values for comparison. In Tables III.2, 3 & 4 are given AAS data on elemental concenterations in green vegetable leaves, green leaves often used as flavouring agent and green vegetables respectively. Two of the samples spinach and mint were also analysed by neutron activation analysis and prompt γ-ray analysis methods and their data for 21 elements are given in Table III.5. Iron concentrations were determined by AAS and spectrophotometric methods for comparison. The respective data for all the samples and SRMs are given in Table III.6.

(i) Standard Reference Materials : These were chosen such that their origin is same but developed by different agencies from USA, UK and Vienna.. Out of five SRMs analysed in this study, Bowen's Kale has been the most widely analysed world over and best values are available for most elements amongst others. Two SRMs (1572 and

1573) are from National Institute of Standards and Technology, USA and the other two (CRM-331 and CRM-359) are from International Atomic Energy Agency, Vienna. The biological matrix of these SRMs perfectly matches with those of 17 samples analysed in this study. The basic purpose of analysing these SRMs is to ensure quality control and validation of data as well as methodology. A comparison of our data with certified values in Table III.1 indicates that we have not been successful in our aim. However, it is to be emphasized that such SRMs should always be analysed before the analysis of actual samples.

(ii) Accuracy and Precision of Data : A comparison of our AAS data with those of the certified values shows no comparability for most values except for Cu, Fe, Mn and Zn in SRM-1572 and CRM-331 in which cases data are in good agreement. In many of the cases large deviations are observed between the observed and certified values. These may, perhaps, be due to incorrect calibration of the instrument or wrong standards used for calibration of the instrument. Since iron concentrations were in better comparison, we also tried to determine it by other method i.e. spectrophotometrically by and Fe(II)-o-

phenanthroline complex method. These data are given in Table III.6. It is observed that iron concentrations as obtained by two methods (AAS and Spectrophotometric) do not compare at all. Therefore, nothing can be said about the accuracy of elemental concentrations in the samples. In order to estimate precision of the measurements 5-replicate analyses of iron by AAS method were carried out. The data are listed in Table III.7. It is observed that standard deviation is small suggesting good precision of these measurements. Also relative standard deviation (RSD) is 7.06 % only. On the basis of these estimates for accuracy and precision it may be said that though overall elemental data for sample may not be reliable but it definitely indicates relative magnitudes in the respective samples.

(iii) Elemental Concentrations by AAA and PGA Methods : Spinach and mint samples were also analysed for 21 elements by NAA and PGA methods. It is observed from Table III.5 that concentrations of Ca and K in two samples as obtained by INAA and PGA methods are in excellent agreement. Even otherwise these two methodologies are well known for their accuracy, precision and sensitivity. Further

standard deviations in Table III.5 as indicated for several elements, are quite small suggesting better precision of data. Therefore, the overall elemental data may be considered as more reliable.

III.2 ELEMENTAL CONTENTS IN VEGETABLES :

As can be seen from data in Tables III.2, 3 & 4 that in all 11 elements in 17 samples have been determined by AAS. Out of the 11 elements, eight (Ca, Mg, Cu, Fe, Co, Cr, Mn and Zn are essential elements which are required by the human body and other three (Ni, Cd & Pb) are environmental or toxic pollutants. In both cases these are found in significant amounts and their origin in human body may be attributed due to intake of the vegetables and other food items.

However, in case of spinach and mint samples analysed by NAA and PGA methods we have data for 21 elements, most of which are essential except for Br and Sb. Elemental occurrences for individual elements in various vegetable and green leafy samples analysed in this study are discussed here.

The elements determined in this study can be broadly classified into three groups; alkaline earth metals (Ca and Mg), transition metals (Cu,

Cr, Fe, Co, Mn and Zn) and toxic pollutants (Ni, Cd and Pb). Their contents and importance are discussed in following lines :

(i) Alkaline earth metals : As reported in literature, adult human body contains over 1 kg Ca and 25 g Mg on an average, chiefly in the skeleton [19]. Milk and cheese are the principal dietary sources of Ca but phytic acid reduces its absorption in the body. Mg is a constituent of the chlorophyll molecule and hence most diets contain an excess. Cereals and vegetables contribute approximately two-thirds of daily intake. In addition to its structural role Mg also activates enzymatic process. Ca is required for the formation and maintenance of skeleton and teeth. It is also required for normal contraction of muscles to make limbs move, contraction of heart for its normal function, nervous activity and blood clotting. The richest source of Ca among vegetables are green leafy vegetables. In our studies, we have found the highest concentration in bitter neem leaves (7.02 mg/g) and lowest in the hybrid of cauliflower and broccoli (0.09 mg/g). In case of green leafy vegetables spinach, fenugreek, radish and gram, it is found in the range of 4-6 mg/g. Growing children need relatively more calcium than adults to meet their requirements.

Insufficiency of Ca may lead to brittleness in bones and teeth. It is a common knowledge that green leafy vegetables are often recommended to children and sick persons. Calcium plays a key regulatory role as a messanger in signal transaction, notably in nerves and muscle cells [68].

Mg shares many properties of Ca as it is also present in bones and helps in absorption, metabolism, tissue distribution. Also, it has a role in cardiovascular diseases. In a recent study Fernandes et al. [69] have correlated Mg deficiency to coronary heart disease and pathophysiologic mechanisms implicated in acute coronary events. Also Darzynkiewicz et al. [70] have investigated the effectiveness Mg supplementation as a means of assisting the traditional therapeutic methods for psychosomatic disturbances. We have observed a uniform distribution of Mg in the range of 0.6 - 0.8 mg/g in all the samples except in cauliflower and broccoli where it is very low : 0.17 and 0.14 mg/g respectively. Thus Mg lies on the border between the macro and micro elements. It is primarily an intracellular element and it exerts regulatory and catalytic roles in many biochemical systems.

(ii) Transition Metals (Cu, Cr, Fe, Co, Mn, Zn) : As expected by the low concentrations, trace elements serve primarily catalytic functions in cells and organisms. Iron performs both major and minor functions. Its deficiency causes anaemia, providing clear evidence of essentiality, but it is also a component of numerous proteins that exert critical role in energy metabolism, notably the cytochromes and the enzymes that participate in the electron transport system [71].

Fe is an essential element for the formation of haemoglobin of red blood cells and plays an important role in the transport of oxygen. It also promotes oxidation of fats but iron (II) and iron (III) differ in their effects, former being more active than the latter to the oxidation of fats. Tissues require iron for various oxidation reduction reactions. Iron deficiency may lead to anaemia and haemorrhage. Rich sources of Fe among vegetables are green leafy vegetable. It is observed from our results, that highest concentration of iron is found in gram leaves (1149 μg/g) followed by spinach (626 μg/g), coriander (465 μg/g), and singhri (570 μg/g) all of which have more than 500 μg/g. However, broccoli has the lowest iron content (113 μg/g). The diet rich in iron is able to meet out our daily iron

requirement and prevent iron deficiency, but not much effective in correcting anaemia. In such cases, medicinal iron in the form of iron salts and other haematinics have to be provided to correct anaemia. In view of widespread prevalence of iron deficiency anaemia in many parts of the world, fortification of foods with iron is advocated to prevent iron deficiency. In our country fortification of common salt with iron have been successfully developed [1].

Zn is an important element performing a wide range of functions in the body as it is a cofactor of a number of enzymes. Zn deficiency leads to growth failure and the appearance of lesions. During past two decades several workers have investigated the zinc deficiency in human subjects. It is reported that Zn deficiency may be correlated with chronic malabsorption state and its measurements may be useful in acute conditions [68]. The human body contains about 2-2.5 g of zinc, approximately 55% of this being located in muscele and 30% in bone, with the rest fairly evenly distributed and the other tissues [71]. From the perusal of the Tables III.2, 3 & 4, we deduce that spinach contains the

maximum amount of zinc (182 μg/g) and the leaves of bitter neem contains the minimum amount (21.2 μg/g).

Copper plays a role in iron absorption and is also involved in cross-linking of connective tissues, neurotransmission and lipid metabolism. Cu is a constituent of oxidative enzymes like cytochromes. High concentrations of Cu occur in milk and milk products. Cu deficiency may lead to anaemia, change of ossification and nervous system damage. As determined by AAS, Cu concentration in maximum in spinach (15.9 μg/g) followed by gram leaves (12.7 μg/g), mustard (12.7μg/g) and fenugreek. (7.6 μg/g). The lowest concentration of 0.1 μg/g has been observed in Broccoli.

Chromium may be present in foods in different oxidation states. Wet oxidation procedures converts all of the chromium present into Cr (VI), whereas Cr(III) is probably the nutritionally required form. Cr functions as the glucose tolerance factor and is studied in relation to diabetes. Its deficiency may lead to impaired glucose tolerance and indigestion. On the basis of our results, we could say that it is quite uniformly distributed in all the green vegetables.

Manganese is an essential element as it participates in a number of reactions as a component of metallo-enzyme or as enzyme cofactor. It also participates in lipid and carbohydrate metabolism. Its deficiency leads to abnormality in skeletal bone mineralisation, dizziness and deafness. The National Research Council of USA has listed dietary requirements for manganese that range from 20 mg/kg for mature animals to 60 mg/kg for young, rapidly growing animals. Because of the poor absorption of manganese from the diet, these levels greatly exceed the actual available requirements (10 mg/kg) [73]. The highest concentration is found in gram leaves (99.7 μg/g) and the lowest in the cabbage (13.2 μg/g).

Cobalt is a biologically essential element as it is a constituent of vitamin B_{12}. Its deficiency leads to loss of appetite and vit. B_{12} deficiency. Spinach contains the maximum amount of Co (326 μg/g). In several vegetables like radish, gram, mint, capricum, beans, cauliflower, broccoli and hybrid it must be present in very low concentrations that may not have been detected by the AAS.

(iii) **Toxic Pollutants (Ni, Cd and Pb) :** Though nickel though is a biologically important element helping in iron absorption and activating enzymes but if present in large amounts becomes toxic. Its highest concentration has been found in spianch (13.6 μg/g) and lowest in broccoli (0.9 μg/g). In all other samples it is distributed uniformly at ~ 1 μg/g.

Cadmium is potentially toxic and a major environmental contaminant. Environmental sources of cadmium are largely from industrial exploitation especially near busy roads, water, plant and sewage sludge. Although cadmium is only partially absorbed by the body, it accumulates in body tissues, mainly in the kidneys. Hence, there is increasing concern over cadmium pollution and its impact on environmental ecosystems. The levels of cadmium present in many foods are, in general, close to or below the limit of detection, i.e., less than 0.01 μg/g . It is found to be maximum in mustard (20.3 μg/g) and lowest in cauliflower and broccoli (1.7 μg/g).

Lead is well known for it toxic nature and a major environmental contaminant from vehicular emission. It is also an industrial hazard.

The exhaust of vehicles is the major cause of its contamination. Man ingests lead from food and drink and inhales it from aerosol and particulate form from the atmosphere. It is found in maximum amount in fenugreek, cabbage and bathua (11 μg/g) but lowest in broccoli (1 μg/g). Main source of these toxic heavy metals may be from the spray of pesticides/insecticides and heavy vehicles on the roadside along the fields. An exhaustive monograph by Ray [76] describes the source of pollutants in foods. It has been suggested that one way of getting rid of heavy pollutants in food is that these should be cleaned thoroughly before cooking.

III.3 MOISTURE AND ASH CONTENT :

A perusal of our data on moisture and ash contents in Table II.1 shows these to be in the range of 76.9 - 93.8% and 6.5 - 21.1% respectively. Since, these are in close range, average contents were calculated; moisture - 88.4 $\pm$ 5.2% and ash - 9.65 $\pm$ 4.64% . For many of the vegetables analyzed in this study moisture content is reported by Gopalan et al [1] and our values are in good agreement. As far as the ash content is concerned, it concentrates the elements on one hand but at the same time many volatile elements or its salts are lost because of heating at more than 500°C in muffle furnace. Several workers [19] have analyzed ash content of the vegetables for elemental contents but these really do not represent correct picture of elemental concentrations. In order to avoid such problems, several alternate procedures have been suggested such dry ashing at low temperature (< 250°C) and/or leaching with mineral acids. Anyhow, moisture and ash contents are also important parameters in elemental analysis and should always be reported.

III.4 MÖSBAUER SPECTRAL STUDY OF SPINACH :

Since iron is an important constituent of green vegetables we thought of studying Mössbauer spectrum of spinach so as to ascertain its oxidation state and bonding aspects. Room temperature Mossbauer spectrum of spinach using ^{57}Co(Rh) source and a constant acceleration mode spectrometer (Wissel) is shown in Fig. III.1. It was recorded by Prof. M. Katada at the Tokyo Metropolitan University,

Japan. A two line spctrum is observed with isomer shift, (δ)=0.31 mm/s (w.r.t. α-iron) and quadrupole splitting, (ΔE_Q)= 0.62 mm/s.

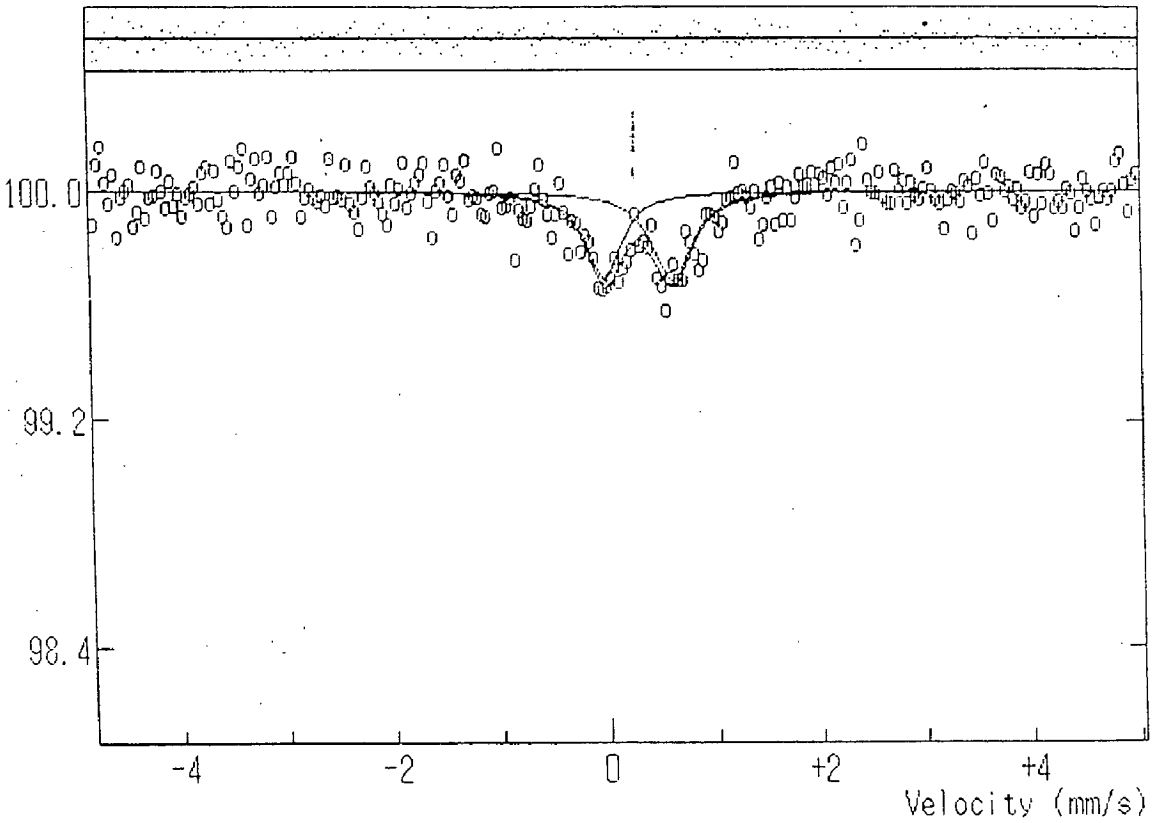

Fig. III.1 Mössbauer Spectrum of spinach at room temperature

Line widths were τ_1 = 0.38 and τ_2 = 0.43 mm/s. As can be seen spectral intensity is very small which is indicative of small amount of iron in the sample under study. Mössbauer studies of a large number of biological molecules (specially home proteins) have been studied in detail and several reviews [74,75] have appeared in literature. These parameters suggest Fe^{3+} in high spin state which may correspond to ferredoxin (Fd II) and these are comparable to that for the $[S_2MoS_2Fe(PhS)_2]$ an iron-sulphur-molybdenum protein often found in plants. These are involved in oxidative electron transfer processes in many different kinds

of functions, e.g., photosynthesis, nitrogen fixation, respiration etc. In this case iron may be as spin-coupled cluster and not an isolated ferric ion. On comparison with literature studies [75], it is suggested that spinach may contain a novel three iron centre protein with magnetic properties. Further studies are in progress.

III.5 ELEMENTAL INTAKE FROM VEGETABLES :

As already pointed out food is the main source of elemental intake. Earlier, it has been suggested that an average man consumes about 200 g leafy and other vegetables [76]. Since moisture content of most leafy vegetables is approximately 90% as per our observation in Table II.1, the amount of dried vegetables may be estimated to be about 20 g. On the basis of our data for often consumed vegetables such as spinach, fenugreek, cabbage, beans and cauliflower in Tables III.2, 3 and 4, average elemental contents for essential elements are found to be for Ca - 2.27 mg/g, Cu - 6.7 μg/g, Fe - 173 μg/g, Cr - 21.7 μg/g, Mn - 26.0 μg/g and Zn - 64.5μg/g. Thus, daily intake of these elements is found to be : Ca - 45.4 mg, Cu - 134 μg, Fe - 3.46 mg, Cr - 434 μg, Mn - 520 μg, Zn - 1.29 mg. On the basis of these calculations and comparison with Recommended Dietary Intakes for Indians [7], it may be said that vegetables contribute significantly as far as the intake of essential elements are concerned.

Table III.1 : Elemental Concentrations in Biological Standard Reference Materials by AAS

Element	Bowen's Kale [62]	SRM-1572 Citrus leaves[63]	SRM-1573 Tomato leaves [64]	CRM-331 Spinach[65]	CRM-359 Cabbage [66]
Ca (mg/g)	8.86 (41.06)	10.98 31.52	7.17 (30.0)	3.78 (15.6)	0.44 (19.12)
Cd (μg/g)	18.2 (0.89)	16.2 (0.03)	10.5 (3)	16.2 (-)	18.2 (0.12)
Co (μg/g)	N.D.	N.D.	N.D.	0.331 (0.382)	0.186 (0.121)
Cr (μg/g)	57.6 *0.369)	17.5 (0.8)	13.5 (4.5)	45.6 (1.92)	17.5 (1.30)
Cu (μg/g)	22.1 (4.89)	16.9 (16.5)	10.7 (11)	11.7 ( )	9.6 (5.52)
Fe (μg/g)	779 (119.3)	642 (90)	489 (690)	329 (293)	859 (154.7)
Mg (mg/g)	0.64 (1.60)	0.79 (5.8)	0.79 (7.0)	0.81 (9.22)	0.64 (2.02)
Mn (μg/g)	53.9 (14.82)	21.8 (23)	144.9 (238)	76.8 (76.2)	32.5 (31.34)
Ni (μg/g)	64.8 (0.895)	20.9 (0.6)	4.7 (-)	4.7 (-)	2.6 (1.31)
Pb (μg/g)	47.2 (2.49)	29.1 (13/3)	1.2 (6.3)	4.9 (-)	41.2 (1.05)
Zn (μg/g)	129 (32.29)	33.2 (29)	44.9 (62)	59.4 (80.3.)	44.9 (57.9)

In parenthesis are given certified values from literature

Table III.2 : Elemental concentrations in green leafy vegetables as determined by AAS

Element	Spinach	Fenugreek	Mustard	Bathua	Radish	Gram
Ca (mg/g)	4.87	4.83	1.46	1.21	4.57	5.96
Cd (μg/g)	18.3	10.5	20.3	2.2	18.3	26
Co (μg/g)	325.5	60.8	48	59.4	N.D.	N.D.
Cr (μg/g)	25.5	21.5	21.5	21.5	25.5	17.5
Cu (μg/g)	15.9	7.6	12.7	5.5	7.6	12.7
Fe (μg/g)	626	313	200	168	385	1149
Mg (m/g)	0.81	0.60	0.78	0.78	0.74	0.78
Mn (μg/g)	56.1	22.5	43.2	27.5	53.2	99.7
Ni (μg/g)	13.6	1.1	4.7	4.7	1.1	2.6
Pb (μg/g)	4.9	11	4.9	11	4.9	4.9
Zn (μg/g)	182	19.2	49.8	122	38.3	36.1

Table III.3: Elemental Concentrations in green leaves often used as flavouring agent and cabbage as determined by AAS

Element	Neem (bitter)	Neem (sweet)	Mint	Coriander	Cabbage
Ca (mg/g)	7.02	6.14	2.73	2.82	1.08
Cd (μg/g)	18.3	10.5	18.3	16.3	18.3
Co (μg/g)	45.2	46.6	N.D.	76.6	184
Cr (μg/g)	17.5	21.5	21.5	13.5	21.5
Cu (μg/g)	6.5	8.5	5.4	9.6	1.3
Fe (μg/g)	208	281	265	466	313
Mg (m,g/g)	0.75	0.77	0.74	0.78	0.61
Mn (μg/g)	21.1	15.4	24.7	40.4	13.2
Ni (μg/g)	1.1	4.7	1.1	1.1	1.1
Pb (μg/g)	4.9	4.9	4.9	4.9	11
Zn (μg/g)	21.2	30.7	26.5	49.1	23.2

Table III.4 : Elemental concentrations in green vegetable leaves as determined by AAS

Element	Capsicum	Beans	Singhri	Cauliflower	Broccoli	Hybrid
Ca (mg/g)	0.65	0.13	3.29	0.23	0.44	0.09
Cd (μg/g)	8.5	10.5	N.D.	1.7	1.7	18.3
Co (μg/g)	ND.	N.D.	45.2	N.D.	N.D.	N.D.
Cr (μg/g)	13.5	21.5	21.5	18.3	26	17.5
Cu (μg/g)	10.7	8.6	6.5	0.2	0.1	6.5
Fe (μg/g)	288	321	570	210	113	152
Mg (m/g)	0.64	0.74	0.73	0.17	0.14	0.64
Mn (μg/g)	17.5	19.7	19	18.7	25	19.7
Ni (μg/g)	1.1	4.7	4.7	N.D.	0.9	4.7
Pb (μg/g)	1.2	4.9	4.9	5.0	1.0	4.9
Zn (μg/g)	32.4	44.5	84.5	53.6	36.3	32.4

Table III.5 Elemental Concentration In Spinach And Mint As Determined By NAA And PGA Methods

ELEMENT	SPINACH		MINT	
	INAA	PGA	INAA	PGA
Au(ng/g)	2.8	N.D.	12	N.D.
B(μg/g)	N.D.	20 ± 3	N.D.	26 ± 1
Br(μg/g)	12.7 ± 2.5	N.D.	7.86 ± 0.37	N.D.
Ca (μg/g)	10.2 ±0.1	13.7 ± 2.3	11.3 ± 0.3	13.3 ± 2.2
Cl(mg/g)	N.D.	13.7 ± 0.2	N.D.	2.18 ± 0.07
Cr(μg/g)	0.54 ± 0.03	N.D.	0.48 ± 0.02	N.D.
Cs(μg/g)	N.D.	N.D.	0.15	N.D.
Cu(μg/g)	83.8 ± 3.3	N.D.	2.02 ± 0.35	N.D.
Fe(μg/g)	1438	N.D.	535	N.D.
K(mg/g)	45.1 ± 1.5	45.1 ± 1.1	91.3 ± 2.6	20.0 ± 0.6
La(μg/g)	0.39	N.D.	0.91	N.D.
Mo(μg/g)	1.03	N.D.	1.82	N.D.
Na(mg)	48.5 ± 5.8	22.7 ± 6.2	0.37 ±0.06	N.D.
Rb(μg/g)	12.3	N.D.	16.6	N.D.
Sb(ng/g)	26	N.D.	150	N.D.
Sc(ng/g)	29 ± 2	N.D.	139 ±18	N.D.
Se(μg/g)	N.D.	N.D.	N.D.	N.D.
Zn(μg/g)	323 ± 14	N.D.	90.8 ± 6.9	N.D.
B(μg/g)	N.D.	20 ± 3	N.D.	26 ± 1
Cl(mg/g)	N.D.	13.7 ± 0.2	N.D.	2.18 ± 0.07
H(mg/g)	N.D.	5.0 ± 0.3	N.D.	57.2 ± 0.3
S(mg/g)	N.D.	4.78 ± 0.62	N.D.	2.85 ± 0.47

ND means not detected

Standard deviations were calculated on the basis of multiple photopeaks and multple countings at different intervals.

Table III.6 Comparison of Fe concentrations as determined by AAS and Spectrophotometric methods

Sample	AAS (μg/g)	Spectrophotometric (μg/g)
Bowen's Kale	779	119.5(119.3)
Citrus leaves(SRM-1572)	642	142 (90 ± 10)
Spinach (CRM-331)	329	335 (293 ± 6)
Tomato leaves (SRM-1573)	490	106 (690 ± 25)
Cabbage (CRM-359)	859	174 (154)
Neem (Sweet)	280	56
Neem (bitter)	208	610
Spinach	626	116
Cabbage	313	89.2
Bathua	168	48.2
Mustard	200	55.5
Hybrid	152	62.5
Coriander	466	99.7
Fenugreek	313	74
Beans	321	60.7
Gram	1149	236
Singhri	570	122
Capsicum	288	65.5
Radish	385	85.5
Mint	264	71.5

In paranthesis are given certified values

Table III.7 Replicate analyses of iron in spinach (CRM-331) as determined by AAS on different days

S.No.	Amount (μg/g)
1	329
2	382
3	365
4	399
5	378

Mean	370.6
Standard Deviation	± 26.2
R.S.D. (%)	7.06

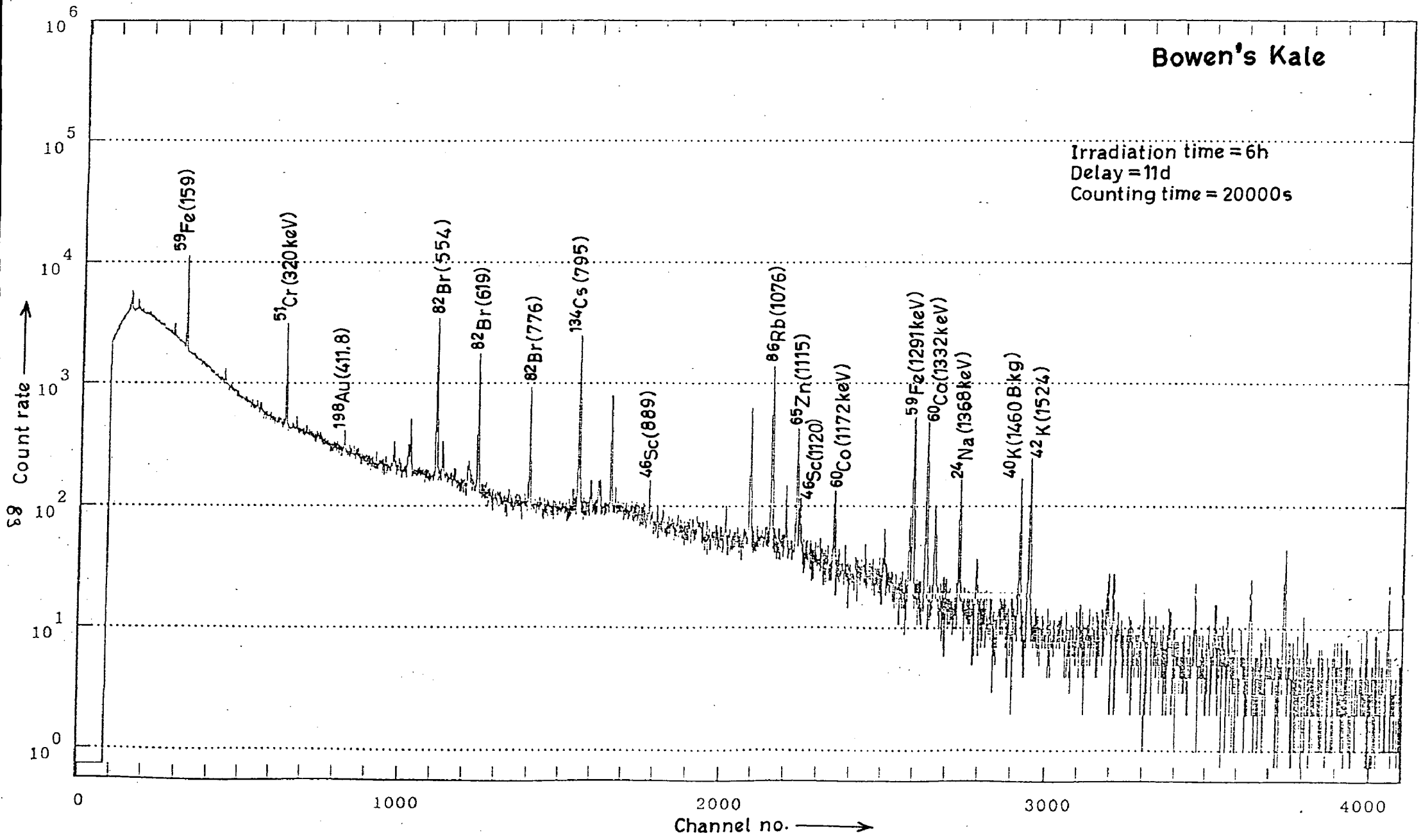

Fig. II.1 Gamma ray Spectrum of Bowen's Kale

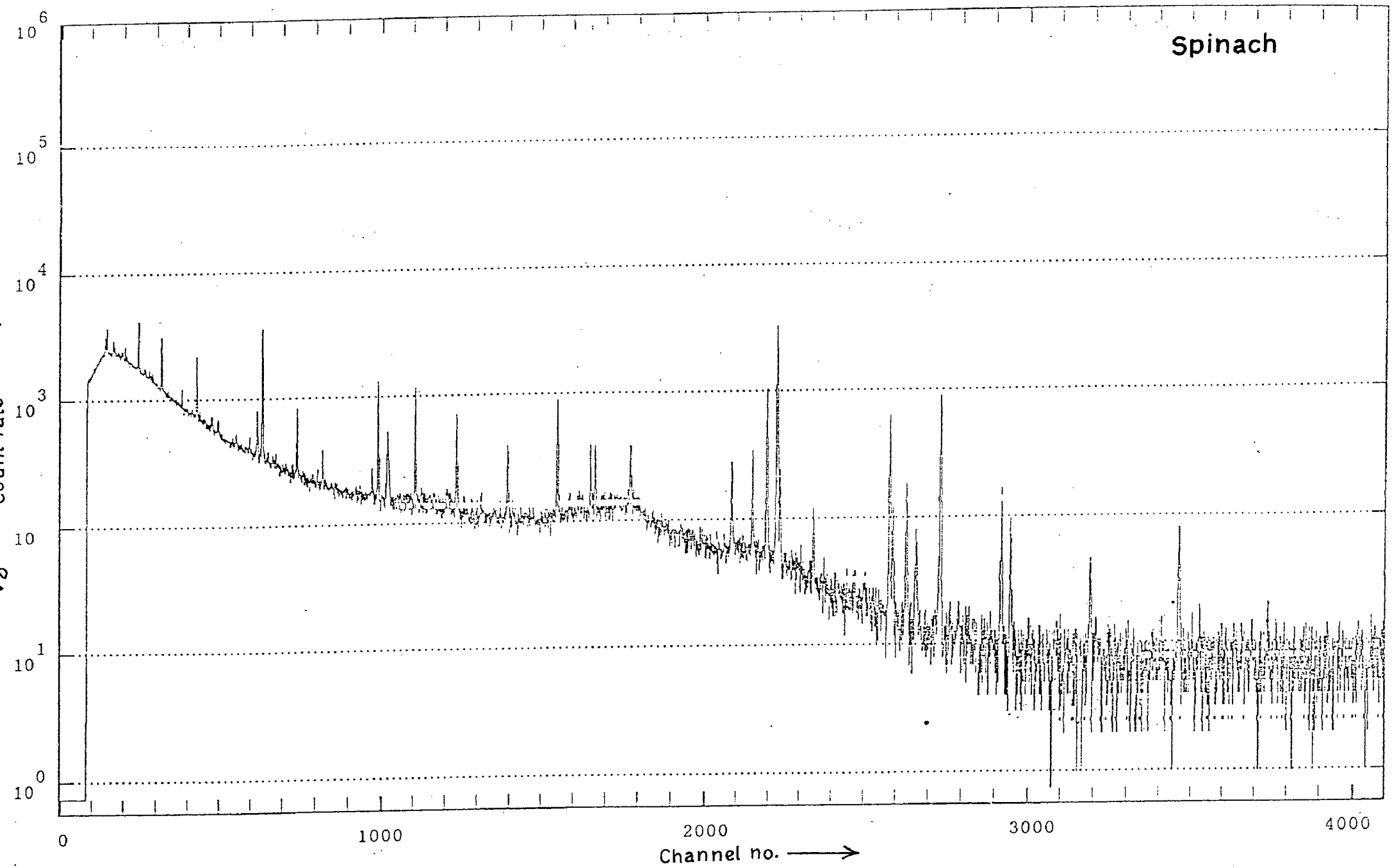

Fig. II.2 Gamma ray Spectrum of spinach

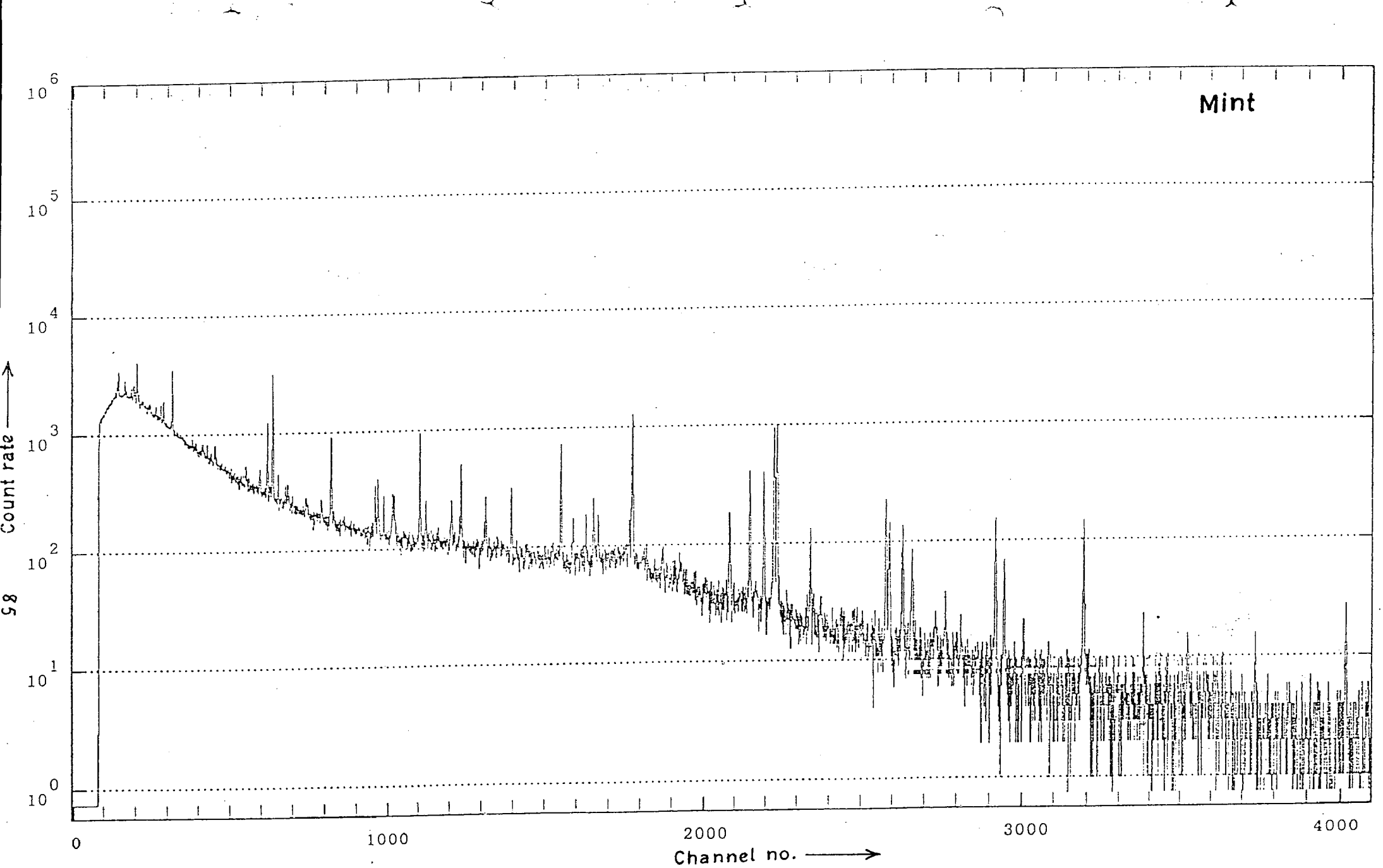

Fig. II.3 Gamma ray Spectrum of mint

CONCLUSION

Analysis of several green leafy vegetables by AAS has shown varying contents for nutritionally essential (Ca, Mg, Cr, Mn, Fe,Co, Cu and Zn) and environmentally toxic (Ni, Cd and Pb) elements. Experimental data for five botanical Standard Reference Materials, analysed for quality control, have shown our data to be of limited importance only. It is observed that only relative magnitudes of elemental contents may be of some relevance. Some green leafy vegetables such as spinach, fenugreek, bathua etc. have been found to be enriched in several essential nutrients whereas, in general, broccoli and cauliflower or their hybrid have very small amounts of these elements. Presence of heavy metal toxic pollutants (Cd and Pb) may be attributed to the use of pesticides/insecticides and vehicular emissions from the road side along the fields. Mossbauer spectral study of spinach has shown iron to be Fe^{3+} in high spin state and comparable with ferredexin (Fd II). A detailed study of elemental contents in dietary components is essential to estimate elemental intake through diet.

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