SPECTROSCOPIC STUDY OF THE TRACE ELEMENTS ON RICE (ORYZA SATIVA. Linn) IN MEGHALAYA

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By
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This is to certify that the thesis entitled "Spectroscopic Study Of the Trace Elements On Rice (oryza sativa Linn) in Meghalaya" submitted to the North Eastern Hill University by Mr. Paul Sansan Dkhar, Institute of Self-Organising Systems and Biophysics, NEHU, for the fulfilment of the degree of Doctor of Philosophy, embodies the record of the original investigation carried out by him under my supervision. He has been duly registered, and the thesis submitted is worthy of being considered for the award of Ph.D degree.

This thesis or any part thereof has not been submitted for any other degree to any other University or Institution.

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COURSES	GRADES
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2. Methods of Science.	O
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CHAPTER-I

INTRODUCTION

1.1 TRACE ELEMENTS IN DIFFERENT FIELDS OF SCIENCE

Trace elements are referred to those elements which are present at trace concentration levels of parts per millions (ppm) or parts per billions (ppb) in a variety of matrices. With the development of Instrumental systems like Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), the degree of measurement of these elements is enhanced upto the level of parts per trillion (ppt) without much difficulty. Though these elements often seem to be very insignificant, they do have an important role in life. The chronological order of the discovery of the essential requirements for a few trace elements is shown in **APPENDIX-I** [1]. The characteristic properties of a complex system and many interesting problems that arise in different spheres of science are derived or can be explained from the absence or presence of specific elements at these low levels of concentration. It is now well recognised that the presence of trace elements in various matrices has attained a fundamental importance in many fields of science, such as Agricultural science, Material science, Medical science, Environmental science, Geological science, Forensic science, Catalyst characterisation and Petroleum products. Consequently, there has been an increasing demand in the detection and assay of these elements. Hence, the study of trace elements has not only evinced the interest of researchers in different fields but also brought together the scientists of many disciplines.

To carry out the trace element analysis, an intensive activity in the investigation of methods of analysis has been evolved. The use of these methods which adopt various analytical techniques for analysis depend on [a] elements of interest [b] chemical characteristics of the sample [c] degree of precision and accuracy necessary for the final analysis and [d] sample availability. A host of techniques varying in degree of success and convenience has emerged. Spectroscopic techniques like Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma- Optical emission Spectroscopy (ICP-OES) have been widely

used for this purpose and have proven to be widely successful. X-Ray Fluoressence (XRF), Neutron Activation Analysis (NAA), Proton Induced X-Ray Emission (PIXE), Charge Particle Activation Analysis (CPAA) are among the other popular techniques being used.

To highlight the wide ranging importance of trace elements, their role in different fields of science are summarised below:-

MEDICAL SCIENCE:- Analysis of trace elements in various organs under normal and pathological conditions is useful in identification of several diseases. Many therapeutic drugs in Ayurvedic system of medicine are administered to a patient as food supplement to treat various ailments. These drugs contain a large number of trace elements and among them those generally considered to be toxic are Hg, Cd, Pb, and As. Proper standardization of these drugs is essential. Inadequate nutriture of Zn, Cu and Fe lead to the alteration of immuno competence in both human and experimental animals [2]. A person suffering from diabetics is known from the concentration of trace elements like Zn, Cr, Mg and Mo. Their amounts in serum are remarkably lower compared to a normal person while Zn in urine is significantly higher [3].

ENVIRONMENTAL SCIENCE:- The importance of trace element analysis is fully recognised when the general public become concerned about the environment and aware of the serious effects of air and water pollution. Once polluted, it becomes a direct threat to the life of both human beings and other organisms [4]. There is an urgent need to initiate more comprehensive action to combat environmental pollution.

Estimating the concentrations of metals in drinking and ground water is extremely important for proper assessment of the hazards associated with their intake [5, 6]. Air pollution is becoming an increasingly serious problem and is in large part a direct consequence of modern Technology. Some metals like Zn, Fe, Mn, Pb, Cr, Cd, Cu in the ambient air affect the human beings mainly through the respiratory tract which may lead to some genetic, physiological, behavioral and psychological disorders and even to death. It was reported that

exposure to Mn in the atmosphere might be linked with several types of respiratory diseases, heart diseases and cancer [7].

GEOLOGICAL SCIENCE:- Analysis of trace elements in geochemical materials has been reported xtensively. The study of trace elements, especially the immobile ones for ancient rocks, has helped the Plate-Tectonic theory which has revolutionised the basic concepts in earth science system to stand the test of time over the years. Chemical composition and trace elements (REE) analysis in the Jiangsu province, China indicated that trace elements enrichment and long term depletion of radiogenic isotopic composition of Sr and Nd were incompatible [8]. The Zawar Pb-Zn deposit of the District of Udaipur, Rajasthan was classified as carbonate-hosted strata bound deposit. The reported analysis of this deposit for trace elements content was shown to be rich in Ag, Fe, Co, Ni but poor in Cu [9].

MATERIAL SCIENCE:- Extensive research in this field has been made especially in steel industry. The determination of trace and ultra- trace levels of Bi is a critical concern because the element has deleterious effects on certain alloys. The need for highly accurate Boron determinations continues to generate a host of new approaches as observed in the literature. Novel approaches to Boron determination in steel reflect the perceived importance of this field of study. Similarly, trace and Ultratrace determination of lead impurities is another concern in the steel industry [10].

CATALYST CHARACTERISATION:- Analytical characterisation of catalysts, heterogenous and homogenous, occupies the central position in catalysis research, both in academia and in industry. In recent years, emphasis has been stressed in attempting to gain a detailed structural picture of the catalysts themselves. Utilising this information, better understanding as to the function of the catalysts has developed which provides guidance in synthesising new and improved catalyst. It was reported that analysis of oxide preparations for the Co-Mo/alumina catalyst showed that an apparent Mo coordination decreases as Mo loading increases. Distortion of Octahedral coordination of the Mo was inferred from the edge

structure and found to parallel the measurements of Mo coordination number. The study confirmed that the distortion decreased by addition of Co [11].

FORENSIC SCIENCE:- Trace element analysis in Forensic science has become a routine analysis which helps in the investigation of crime committed by individual suspects. The analysis of several trace elements in shot gun pellets is carried out for this purpose. The determination of Sb from a shot gun pellets is consistent with an individual having just fired or handled a firearm. The distribution of Sb around a shot gun hole can be evaluated for firing distance determination. Trace elements like Mn, Ba, Pb and Sb in shot gun residue (SGR) on individual hands are determined to give a better clue [11]. It is reported that Pb concentration in hair determines the age of the person [12, 13].

FOOD ANALYSIS:- Trace elements determination in food is also important to ascertain the presence of toxic elements such as As, Hg, Pb, and Se which may be harmful for human health when consumed. The determination of metals like Pb, Cd, Zn, Cu, Fe, Cr and Ni in foodstuffs is an area of interest to some workers [14]. Trace element concentrations of elements like Fe, Ni, Cu, Zn, Sr and others were reported to have been determined in fish from the Bay of Bengal [15].

PETROLEUM PRODUCTS:- Trace elements in petroleum have received increasing attention in the recent years owing to their importance in understanding problems associated with environmental pollution, petroleum geochemistry, characterisation studies, design and operation of refineries, petroleum migration, and oil spill identification. Petroleum which contains large amounts of hydrocarbons also contains large number of trace elements such as Cu, Co, Cr, V, Ni which significantly influence the performance haracteristics of the end product. Other trace elements such as Sb, As, Be, Ni, Cd, Cr, Co, Pb, Mn, Hg, Mo, Se and V are also potential causes of environmental pollution. Ni and V were determined in fuel oil and crude oil by Wood and Keynes [16] and Ni, Zn, Cu, Na, Pb, Cd, and Fe in petroleum crudes by Osibanjo et al [17].

SOIL SCIENCE:- The estimation of some trace elements in the soils is essential as soil is by far the most important medium that supports plant growth and also plays a key role in crop production. Trace element analysis is extensively used as one of the diagnostic tools in monitoring nutrient status of the soil for fertility. The study of trace element is important because if a trace element is judiciously applied, it acts as a profitable fertilizer, otherwise it acts as a pollutant. If soil is low in an essential nutrient, plants may suffer from deficiency of this nutrient. Non-availability of the trace elements in the soil may result in the manifestation of specific symptoms on the plant parts. Zinc deficiency in rice plant was first described in India and was found to be wide spread throughout Asia [18].

PLANT SCIENCE:- Plant needs a very small quantity of certain trace elements to complete their life cycle. Trace elements occur in plant protoplasm in exceedingly small amounts and each of them plays a specific role for the growth and development of the plant. Several trace elements are involved in the production of chlorophyll, in oxidation-reduction process and in enzymes systems of plant. A deficiency of any of these trace elements cause some abnormal conditions and upsets the growth of the plant. The threshold value of trace elements vary not only with the crops of different species, but also with crops of different varieties of the same species. Lidon and Henriques [19], reported that copper toxicity in rice plant causes the reduction of root length, which appears to be a direct result of the accumulation of excess copper in the total tissue. Some trace elements function in the enzyme systems of plant and among them, copper acts as cation-forming element which is more likely to serve as coenzymes that activate an enzyme but is not an integral part of the molecules. Hence, the right amount of the nutrient elements must be applied, and it must be uniformly istributed. Plants suffering from trace element deficiency/toxicity need not show any symptoms at all except in so far as growth is not as good as it might be, or they may only display symptoms for a short period in the growing seasons.

Besides the above fields, there are still many other areas [10, 11, 20] in which trace element analysis can be applied to achieve new findings which may finally lead to the improvement and confirmation of the previous results.

1.2 PRESENT STUDY AND ITS IMPORTANCE

Meghalaya is a hilly state of India and lies between 25°47' N and 26°10' N latitude and 89°45' and 92°47' E longitude. Its main crop is rice and it is also the staple food of the people of this state. Rice is grown in different agro-climatic conditions with altitudes ranging from 80m to 1850m above mean sea level (msl). Due to variations in climatic conditions and land topography, the rice growing areas have been classified into three altitudinal zones namely lower altitude (<700m,msl), medium altitude (701-1200m,msl) and higher altitude (>1200m,msl). The production of rice in Meghalaya is only 1.1t/ha which is below the national average production of 1.9 t/ha [21, 22]. In Meghalaya, the area of rice cultivation is 1.04 lakh hectare while the total area is approximately 22.5 lakh hectare.

There may be many factors which contribute to the low yield of the crop in this state. One of these factors which may be responsibled for the growth of the plant and finally its yield is the poor management of the plant nutrients. Among the elements, there are the major elements like N, P, K, Ca, Mg, C, H, O, S which are used in relatively large quantities and they are called Macronutrients. Other elements like Fe, Mn, Cu, Zn, Mo, and B are required by the plant in very small amount and they are called the Minor elements [23]. The minor elements are also called the Trace-Elements or Micronutrients [24, 25]. All trace elements must be present in optimum amounts and in forms usable by the plant for its growth and development. At high concentrations, these trace elements are toxic to the plant while at low concentrations, they cause deficiency.

The importance of trace elements in soil fertility and plant nutrition has motivated us to undertake the present investigation. This investigation deals with the analysis of trace elements in the soil and their concentration in the plant at different growth stages from

different altitude zones and their interrelationship. Some of the important Physico-chemical properties of these soils such as Texture, pH, organic carbon and Cation Exchange Capacity (CEC) was also studied. Previous workers had tried to analyse few element like Zn, Cu and Mn in the soil but not in the plant [26]. We therefore strongly felt the need of studying the presence of a wider range of trace elements both in rice soils and plants at different growth stages. This will surely help to have a better idea in taking further sudies for possibly enhancing the yield. A comparison of the value obtained with the critical values available helps one to have more insight of the problem. The inter-element correlation studies both in the rice growing soils and plant at different growth stages was also performed.

Trace elements are involved in metabolic processes of plant enzymes which regulate many vital reactions for the growth and development of the plant [25]. It was therefore thought important to study the relationship between the activity of a few metallo-enzymes and the concentration of the trace elements involved.

In our study, thirteen trace elements had been selected to analyse both in rice growing soils and plant at different growth stages. These samples were collected from different rice growing areas of varying altitude. They are B, Al, V, Cr, Mn. Fe, Co, Ni, Cu, Zn, Se, Mo and Cd. Spectroscopic techniques were used for the analysis. The role and effects of these elements either in plant or in man or in both are given in APPENDIX-II.

The main objectives of the study were:-

(1) Physico-chemical properties of the rice soils of Meghalaya.

(2) Status of the trace elements in rice soils of Meghalaya under varying altitude.

(3) Distribution of the trace elements in rice plant at different growth stages

(4) Enzyme activity in rice plant at different growth stages.

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CHAPTER-II

ANALYTICAL TECHNIQUES

Trace element analysis is useful in many fields that encompass eological science, food analysis, forensic science, material science, soil and plant science and in environmental studies. To carry out the analysis, many techniques varying in degree of sensitivity and convenience have been developed. These include Atomic Absorption Spectrometry, Inductively Coupled Plasma-atomic emission Spectrometry, X-ray Fluorescence, Proton Induced x-ray Emission, Neutron Activation analysis and Mass Spectrometry. Atomic Absorption Spctrometry and Inductively Coupled Plasma-atomic emission Spectrometry were the main techniques used here while X-ray Fluorescence was also used for complementary analysis. These techniques are described in this chapter.

2.1 ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

INTRODUCTION

The Atomic Absorption spectra was first observed in 1802 by Wallaston as dark lines in the spectrum of the sun. These lines are the absorption spectra of elements present in the sun. Walsh [1] utilised it for analytical purpose and coined the name Atomic Absorption Spectroscopy (AAS). AAS became a powerful tool for trace element analysis since the early sixties [2]. Further development of this technique has been developed by several workers [3-13]. The technique offers a rapid and specific determination of trace element concentrations with only a few interferences. It is widely used for various analytical purposes. The technique involves the study of the absorption of radiation by neutral atoms in the gaseous state. The sample is first converted into an atomic vapour and then the absorption of selected wavelengths by atomic vapour is measured. The technique is also known as Absorption flame photometry because all

analytical applications of atomic absorption involve spraying a solution of the sample into the flame.

We used Perkin Elmer Model No. 2380 for our measurement. The schematic diagram of the spectrometer is shown in **Fig-1** and **FIG-2.** It is a microprocessor-controlled system which measures the concentration of metallic elements in a variety of matrices. It provides integrated values of absorbance, concentration and emission intensity. The range of integration can be selected over a period varying from 0.2 to 60 secs.

PRINCIPLE OF AAS

If monochromatic light of intensity I_0 and frequency υ passes through the sample cell containing ground state atoms, the atoms can absorb part of the light beam whose frequency matches with the characteristic frequency of the atom. It decreases the intensity of the light. The decrease in intensity depends upon the oncentration of atoms in the flame cell. The light subsequently passes to a detector where the reduced intensity (I) is measured. It is given by Beer's Law.

$$I = I_0 e^{-abc}$$

where I_0 = Initial intensity of light source

I = final intensity

a = absorption coefficient

b = path length of light intercepted

c = concentration of metal atoms

It can be recast in the form

$$\ln I_0 / I = abc$$

Thus the product abc gives the absorbance (A) of light. Since "a" and "b" are constants, specific to the measuring conditions, Absorbance is directly proportional to the concentration. By measuring the absorbance, the concentration of the element can be determined.

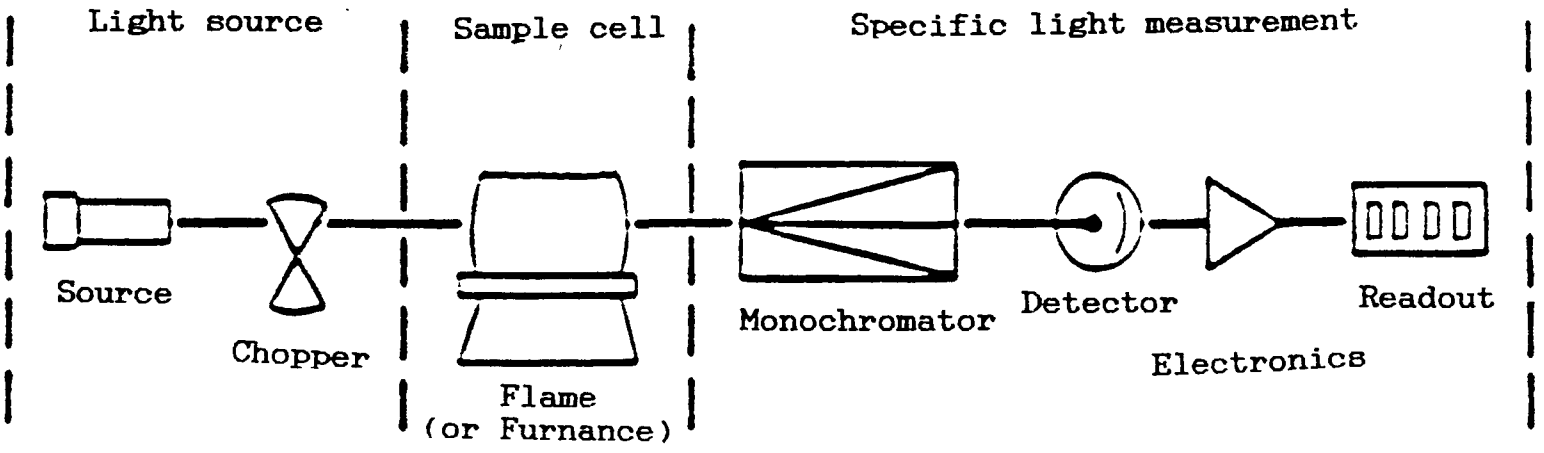


FIG-1. SCHEMATIC DIAGRAM OF A SINGLE BEAM ATOMIC ABSORPTION SPECTROPHOTOMETER

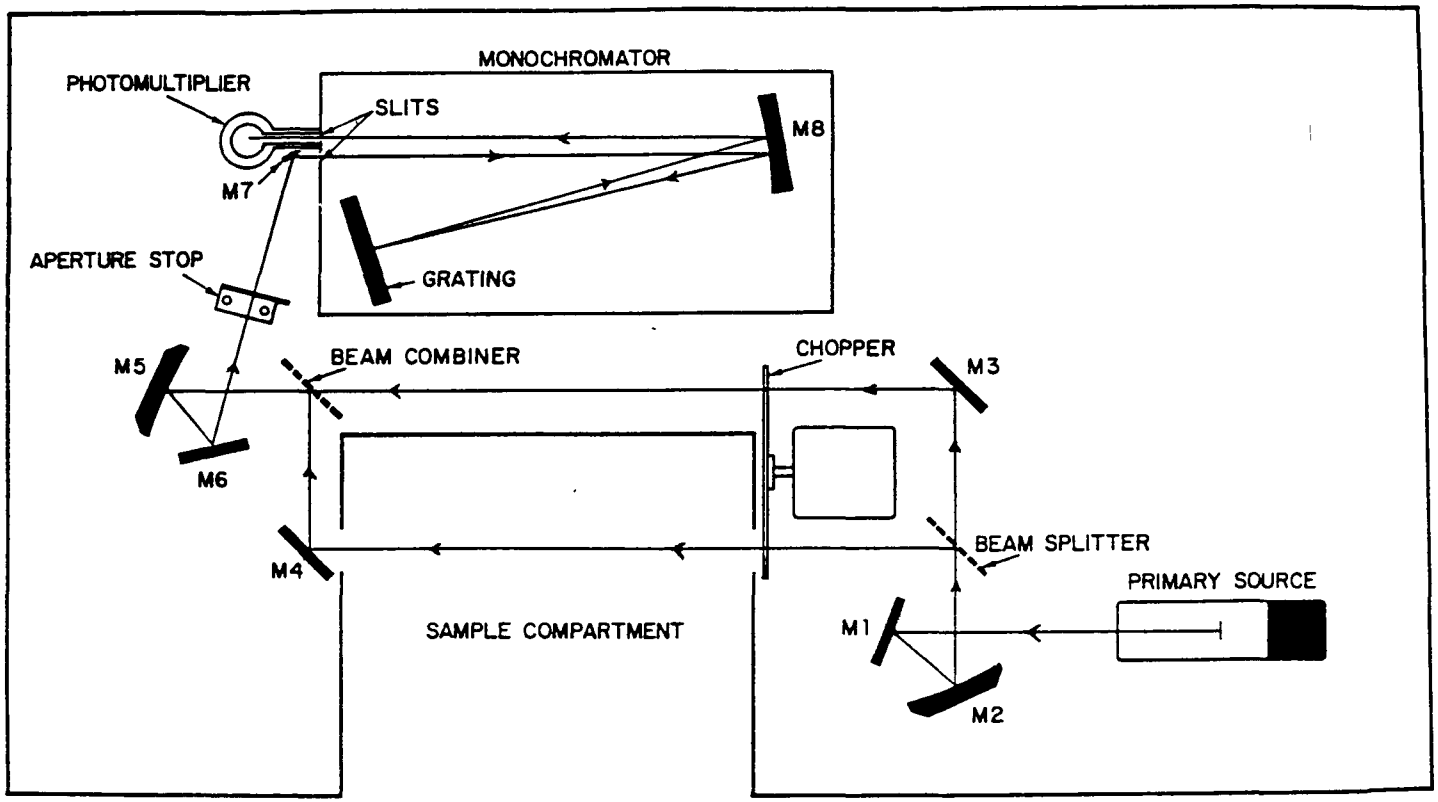


FIG-2. OPTICAL SCHEMATIC DIAGRAM OF ATOMIC ABSORPTION SPECTROPHOTOMETER

CALIBRATION

Quantitative measurements in Atomic Absorption are based on Beer's law, which states that concentration is proportional to absorbance ($c \propto A$). However, for most elements, particularly at high concentation, the relationship between concentration and absorbance deviates from Beer's law and is not linear. The nonlinearity at high concentration is corrected by the built-in microprocessor of the instrument. The microprocessor has the ability to calibrate and compute concentrations using absorbance data even for nonlinear calibration curves. In the linear region, data on one standard and a blank are sufficient for obtaining the relationship between concentration and absorbance. In the nonlinear region, more standards are required. The accuracy of a calibration computed for a nonlinear relationship, depends on the number of standards used. The instrument uses a Patented algorithm (Curve Correction Algorithm). Its principle is given below. The algorithm assumes that the relationship between concentration and absorbance is given by

$$C = \frac{K_3 A^2 + K_1 A}{K_2 A - 1} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

The above equation requires three standards for calibration. The equation can also be used if only two calibration standards are available. One then sets K_3 equal to zero. Similarly if only one standard is available, both K_2 and K_3 are set equal to zero. In actual measurement, a blank is measured first and the autozero operation stores the blank value. This value is automatically substracted from all subsequent readings. Using the first standard for calibration, one stores its absorbance and the value for K_1 is determined by it. When second standard is measured, its absorbance is also stored along with a new value of K_1 and a value of K_2 is calculated using the method of simultaneous equation. Similarly, using a third standard for calibration, new values for K_1, K_2 and K_3 are calculated. The equation (1) is very flexible and it is possible to work with calibration curve ranging from a straight line to an extremely nonlinear one.

Selecting the number and the concentation of calibration standards is very important. One calibration standard (S1) is used when the concentration of the element present in the sample falls within the linear range (< 0.25 - 0.30 absorbance units). Once the concentration of the element present in the sample exceed the linear range, two or three calibration standards are used. The second calibration standard (S2) is approximately 3x the concentration of S1 and the third standard is approximately 2x the concentration of the second standard (S2). The typical calibration curves of few elements Chromium, Zinc and Copper with one, two and three standards respectively are shown in **FIG-3[a, b and c].**

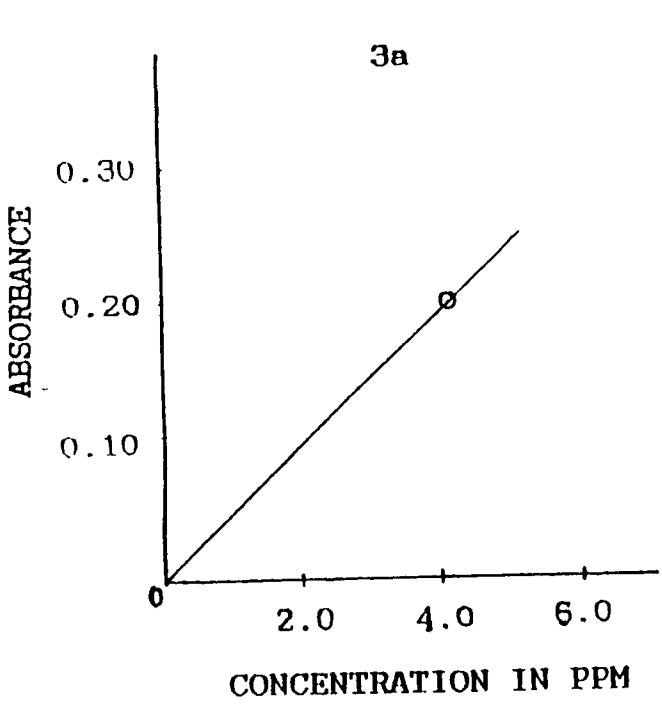
SENSITIVITY AND DETECTION LIMIT

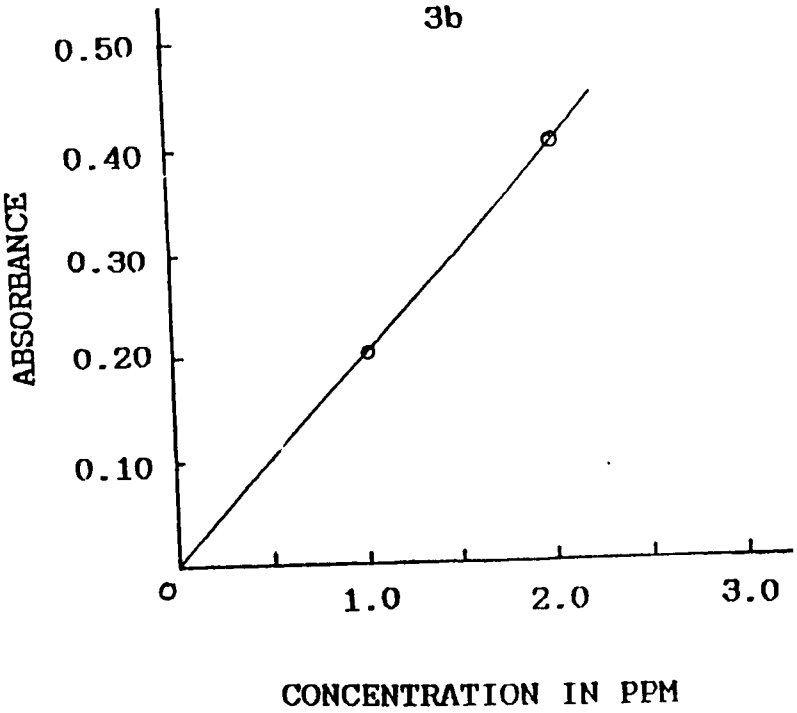
Sensitivity in AAS is defined as the concentration of an element (mg/L) that produces 1% absorption (0.0044 absorbance units) of the signal. The sensitivity for the detection of an element can be determined by measuring the absorbance produced by a known concentration of the element. It is given by

$$\text{Sensitivity} = \frac{\text{Concentration of Std . X } 0.0044}{\text{Absorbance shown by the Std.}}$$

The sensitivity values for different elements at specific wavelength has been supplied by the manufacturer. These are given in **APPENDIX-III.**

Sensitivity check: To have the optimum operating condition, the burner position, nebulizer and gas flow are adjusted till the desired reading absorbance for a particular solution concentration is obtained. It is defined as the concentration (mg/L) of the element at a specific wavelengh that produces a signal of approximately 0.2 absorbance units. The concentration of different elements for sensitivity check is given in **APPENDIX-III.**





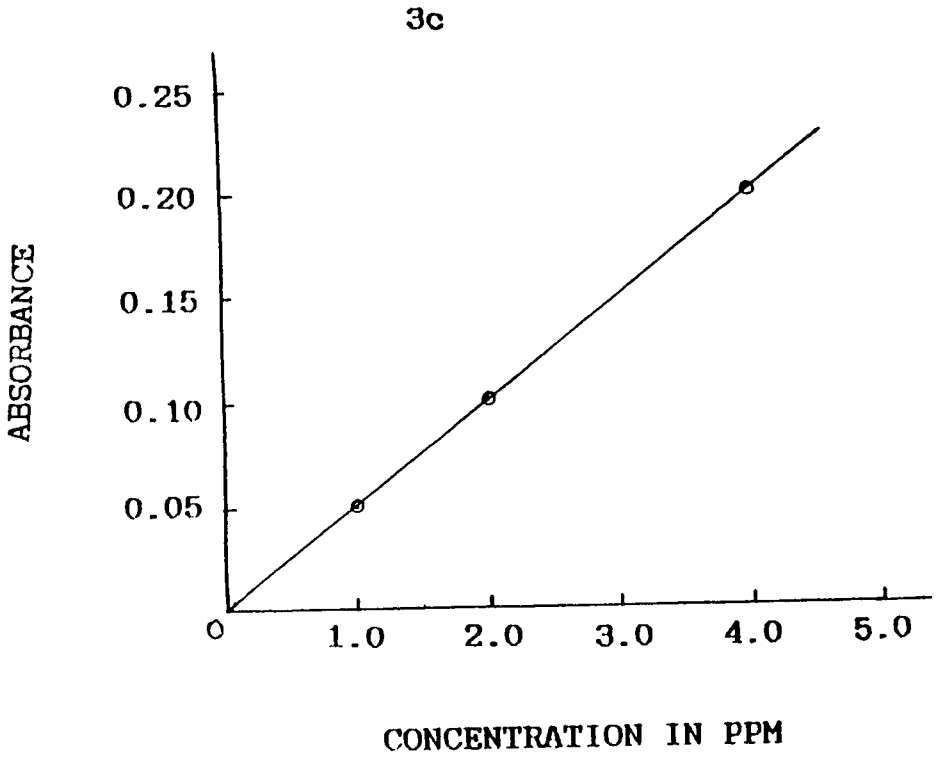


FIG-3 [a, b & c] SHOWS THE CALIBRATION CURVES FOR CHROMIUM, ZINC AND COPPER USING ONE, TWO AND THREE STANDARDS

DETECTION LIMIT

The detection limit is defined as the lowest concentration that is clearly differentiated from zero. We have used a criteria for the detection limit as the concentration of the element which produces a signal/noise ratio of 2. The procedure for determining the detection limit is as follows:-

Two concentrations of the element are prepared, with separate volumetric glassware. It is done to minimise the possibility of contamination. The lower concentration of the standard is made approximately 5X the expected detection limit and the second standard is made twice of the first concentration. After establishing the optimum conditions, the measurements with each standard solution are taken alternately for more than twenty times. A blank reading (solvent only) is made between each standard. The sequence adopted is blank, low concentration standard, blank and high concentration standard. The two blank readings taken immediately before and after each standard are averaged and then the average is subtracted from the standard readings. The mean and standard deviation for the set of corrected high standard readings and low standard readings are calculated. The detection limit is calculated by

$$\text{Detection limit} = \frac{\text{Standard Conc. X 3 Std deviation}}{\text{Mean}}$$

The calculation is made independently for each standard concentration, and the detection limit is the average of the two results.

INTERFERENCES

In the estimation of trace elements the interferences like chemical, ionisation, matrix, emission, spectral and background absorption do exist. These are well understood and methods have been developed either to eliminate or compensate them A brief description of various interferences are given below:

Chemical: This interference occurs when total decomposition of the sample by the flame does not take place It happens if the compound is thermally stable. This is overcome

either by using a higher temperature flame or by adding an appropriate releasing agent.

Ionisation: This occurs when the flame temperature has enough energy to create an ion. It is overcome by the addition of alkali metals like **K, Na, Rb,** and **Cs** to the samples as well as to the standards. The alkali metals have very low ionisation potentials and consequenty they supress ionisation.

Matrix: This interference is caused when physical characteristics of the sample and the standards differ considerably. The large difference in physical characters can either suppress or enhance the analyte signal. It can be minimised by matching the matrix components in the standards with the samples.

Emission: This interference occurs at high analyte concentration when the signal from the highly emissive elements like **Zn, Cd, Se,** falls within the spectral bandpass being used. This degradation occurs because electronic noise of the photomultiplier is proportional to the total signal incident upon it, even though only the modulated atomic absorption signal is being measured. It can be remedied by decreasing the slit width, increasing the lamp current, diluting the sample and using a cooler flame.

Spectral: This interference occurs when an absorbing wavelength of an element present in the sample falls within the bandwidth of the absorption line of the element of interest which is due to the large size of the slit width. Hence by using a smaller slit width, this can be overcome.

Background Absorption: There are two causes of background absorption. **(a)** light scattering particles in the flame **(b)** absorption of light from the lamp by molecules in the flame. To compensate it, a background corrector which utilises a continuum source of Deuterium arc lamp in the UV or a Tungsten-Iodide lamp for visible wavelength is used.

In our analysis, the matrix and emission interferences were only significant. The matrix interference was overcome by matching the martix component in the samples with the standards. This was done by bringing the instrument into the Absorption mode. The unknown sample was aspirated and the value of absorbance was determined Subsequently, the known concentration standards starting from lower to higher

concentration were aspirated till the absorbance value of standards came close to the unknown sample. In case of emission interference, it was minimised by reducing the slit width, increasing the lamp current and diluting the sample.

OPTIMAL OPERATION PROCEDURE

Light Source: The main sources used for Atomic Absorption are the hollow cathode lamp (HCL) and the electrodeless discharge lamp (EDL). The HCL was the source used for our work. HCL is employed as it is an excellent, bright, stable line source for most elements. It provides high intensity and makes Atomic Absorption a specific analytical technique. This is necessary for a source to emit the narrow line spectra of the element of interest as the atoms absorb light at a very specific wavelength. Each HCL has a particular current for optimum performance. In general, higher currents produced a brighter emission and less baseline noise. As the current continues to increase, however spectral line broadening occurs, resulting in a reduction in sensitivity and linear working range. EDLs are used for volatile elements where low intensity and short lamp life time are a problem.

Lamp current: Proper current for the instrument being used was invariably checked. If lamps are operated at the higher current settings, their life can be shortened due to gas being absorbed in the sputtered metal or the glass. Lamp current is to be increased to the maximum value rating only when it is old. For each lamp used, maximum and operating current is supplied. Luckily all lamps worked at the recommended operating current and gave satisfactory results throughout our analysis.

Warm-Up Time: A short warm-up period for about 10-15 minutes is needed for a HCL to reach a constant emission intensity.

Operating Power: The power setting recommended on the label was selected to give optimum performance under most circumstances. At this setting, the lamp gave the maximum intensity consistent with maximum sensitivity. Increasing the power slightly (1/2 to 1 watt) above the suggested value will greatly increase intensity, but may result in reduced analytical sensitivity and possible reduced lamp life. Operating the lamp at a slightly lower power (1/2 to 1 watt) less than the suggested value will not degrade

analytical performance and may extend the life of the lamp as maximum intensity is not required.

Burner Adjustment: To obtain maximum sensitivity, the burner head relative to the light path of the instrument is adjusted by first lowering the burner until the burner head is well below the light beam. Lateral and rotational adjustment of the burner is made with the flame ignited while aspirating a standard solution of the element of interest. Maximum absorption is obtained by adjusting the burner in both cases. The burner used by us is the Dual-Option Burner system which includes both a flow spoiler and an impact bead for optimum operation under different analytical conditions. The A-Ac and N-Ac flames are employed and the burner heads are adjusted accordingly. The burner head was 10cm for A-Ac flame and 5cm for N-Ac flame

Flame: The two most common oxidant/fuel combination used in AAS are Air-Acetylene (A-Ac) and Nitrous oxide-Acetylene (N-Ac). We used both these flames. The temperature of A-Ac is about 2300° C and the flow rate of Ac gas is about 4 litres/min. This fuel is particularly used for the determination of nine elements (**Cd, Zn, Co, Se, Ni, Cr, Cu, Fe, Mn**). The N-Ac flame burns with the maximum tempertaure of about 2900° C and it is mainly used for the determination of three elements (**Al, Mo, V**) which forms refractory oxides. The flow rate of Ac is about 14 litres/min. The high temperature of the N-Ac flame overcomes the chemical interferences.

The fuel/oxidant ratio is adjusted for maximum sensitivity for all elements detected. This is done by aspirating a standards solution and checking to zero after each flow charge and then readjusting if necessary.

The other flame like Air-Hydrogen (A-H_2) and Argon-Hydrogen (Ar-H_2) entrained air are not suitable for our work for they burn with a lower temperature of approximately 2000^0 C and 300^0 - 800^0 C respectively.

Instrument Calibration: Prior to the calibration of the instrument, a sensitivity check was first erformed for each element to be analysed. It is then followed by a proper selection of calibration standards depending upon the concentrations of the elements present in the sample. If invalid calibration is attempted, an error code is displayed.

Wavelength: In our analysis, wavelengths were properly selected up to the accuracy of first decimal place for all the elements as given in **APPENDIX-III**.

Slit width: The list of the prescribed slit width for the detected elements is given in **APPENDIX-III** for optimum operation. Accordingly, the slit width have been adjusted for all the detected elements before our analysis is performed. Infact, other slit widths could be used but it would have affected the sensitivity and signal to noise ratio.

Relative noise: It is used to judge the stability of a particular line. The relative noise is based on the standard deviation (S.D) values measured under specific conditions with the flame on.

Sample preparation: Sample preparation requires crushing the sample in order to have proper homogenisation so that it gives a representative sample. It is then weighed accurately in a Metallic balance (**SHIMADZU Model LIBROR AEG- No. 220**). This gives an accuracy up to the third decimal places (0.001g). Its capacity is 220.000g. For AAS and ICP, the sample has to be in the solution form and this is prepared by using appropriate acids and distilled water.

PRECISION AND ACCURACY

The precision of AAS measurements depends upon two factors:-

(1) Stability of Hollow Cathode Lamp and **(2)** Steadiness of atomisation of the sample solution in the flame.

The fluctuations in the hollow cathode source can be minimised by using double beam instrument. In this case, the light coming from the source lamp is divided into a sample beam, which is focused through the sample cell and the reference beam which is directed around the sample cell. In a double beam system, the read out represents the ratio of the sample and the reference beams. Therefore, the fluctuations in source intensity do not affect the instrument readout and the baseline is much more stable. The double beam spectrometer is not available to us. We have mentioned it for completeness. Besides considering the above two factors, the precision of the instrument can be improved by using longer integration time.

The relative accuracy in AAS is nearly independent of the concentration of the metal in the sample. Hence this technique is much better for the accurate determination of the minor constituents rather than of major constituents.

The factors which affect accuracy are:

1. Weighing: The accuracy of the analytical balance used for weighing the sample is very important. Also the material being weighed should be homogenised properly so as to give a representative sample.
2. Volumetric pipetting: Calibrating pipettes must be used and while diluting the sample, a volume of less than 5.0 ml is to be taken at a time.
3. Volumetric flasks: Volumetric flasks of high grade(class A) are used in order to minimise error in our analysis.
4. Purity of added reagents: Analytical reagents are used and sometimes it is necessary to use ultra-pure chemicals, depending on the analysis.
5. Control of interferences: If matrix interference is present, the standards should be matched as close as possible with the samples.
6. Standards: Available stock standard solution are checked first to make sure that all standards used are made up properly. Then from these standards, solutions of different concentration are made by taking special care in making up the volume required.
7. Calibration: Proper number of calibration standards depending upon the concentration of the elements present in the sample is to be used. Moreover, check standards has to be used for enhancing the accuracy of our analysis.
8. Validity of the blank: The blank should be representative of the matrix of the samples which has been digested. The blank should contains all the reagents used in the sample preparation.
9. Cleaniness of premix burner chamber: The burner chamber is to be cleaned at least once a week.
10. Nebulizer: A corrosion-resistant or a platinium-/rhodium nebulizer is used when working with corrosive solutions.

ACCESSORY SAMPLING TECHNIQUES

To increase the sensitivity and sampling efficiency, there are several accessories which can do so. They are Sampling Boat and Delves Cup system, Flameless Mercury Analysis System, mercury/Hydride Systems and HGA Graphite Furnance. In our analysis, the Mercury/Hydride system was used for the determination of Selenium which is the metallic hydride forming element. In Hydride generation, the gaseous hydride of the metal was chemically produced by the addition of Sodium Borohydride. The gaseous hydride and hydrogen produced by the reaction were then swept by an Argon purge into a heated quartz cell. When the sample vapour was atomised in the cell, a peak signal was produced, the height of which was proportional to the amount of the analyte in the sample. A manual Hydride Generation was available for us.

SPECIAL CHARACTERISTICS OF THE TECHNIQUE

The most important advantages of this technique are:-

1. Its high degree of freedom from interference from its environment, i.e. by the presence of other elements. Minute amount of an element can be determined in the presence of high concentration of the others. It also requires only a small amount of the sample; usually 0.1gm is sufficient to detect many elements present in the sample. The volume of solution needed to detect one element in the sample solution is about 0.5ml.
2. It is highly specific, because the atoms of a particular element can absorb radiation of their own characteristic wavelength and hence spectral interferences are decreased.
3. The technique is more sensitive than emission flame photometry and more elements can be quantitatively determined by this technique.
4. It is not sensitive to small changes of flame temperature.
5. Sensitivity is higher for most elements compared to flame emission technique
6. More elements can be qualitatively determined.

Though this technique is a powerful tool for trace element analysis, yet it is not free from some of its drawbacks and these are given below:-

1. The method is destructive.
2. It requires the sample in the liquid form and analysis of only one element is possible at a time. Moreover, it needs a separate lamp source for each element.
3. This technique is limited to metals only. With non-metals which have their resonance lines in the ultraviolet region, difficulties arise from strong absorption of light by the oxygen in the light path and from the flame gases. Also elements such as Al, Ti, W, Mo, Si cannot be detected, because these elements give rise to oxides in the flame.

2.2 INDUCTIVELY COUPLED PLASMA- ATOMIC EMISSION SPECTROPHOTOMETER (ICP-AES)

INTRODUCTION

The ICP-AES is another analytical instrumental which can be used both for simultaneous and sequential determination of elements having concentration levels as low a few parts per billion (ppb). It is applied in different fields like Geophysics, Geochemistry, Environmental studies, Soil and Plant science for quick elemental scanning especially for ultra rapid multi-elemental analysis. The range of determinable elements by this technique is large and a significant part of the periodic table can be covered. The technique is capable of excellent precision and accuracy with the appropriate safeguards. ICP-AES is also used for monitoring impurities in chemicals, food and other manufacturing products.

This technique employs the high temperature of the plasma (10,000° K), and combines with a very high resolution holographic grating. A well developed computer software (Slimpac) is used for determining a wide range of elements having very low detection limits and less chemical interferences. The unique characteristic of this technique enables us to determine most of the refractory elements like V, Ti and Al. Besides the analytical linearity of the ICP far exceeds that of Atomic Absorption. Though the technique is destructive, yet the special features possessed by it, offers great

opportunities and advantages to use it as a spectroscopic technique. The instrument used for our measurement is the **LABTAM MODEL 8440M**.

The importance of ICP as a technique was first introduced by G. I. Babat (14) for studying the properties of electrodeless discharges. His work signified the first successful operation of ICP's at atmospheric pressure. Following this, in the early 1960's Reed used his ingenious approach for the stabilization and thermal isolation of these plasmas. His work highlighted the attractive properties of plasma and these are **(a)** high gas temperature **(b)** capability of being sustained in noble gas environments (important from free-atom lifetime considerations), and **(c)** freedom from contamination from electrodes. Guided by these properties, plasma is used as the source of elemental excitation. Greenfield [15], Wendt and Fassell [16] demonstrated the usefulness of this technique in elemental analysis. The further development of this technique has been contributed by several workers [11-13, 17-26].

PRINCIPLE OF ICP

The ICP spectroscopy makes use of the important property of the plasma that when the plasma is sufficiently ionised, large quantities of electrical energy can be transfered to it whereby it can be heated to very high temperatures. Hence, when a sample is energised in such a sufficiently high temperature environment, the different elements present in it are thus vapourised, atomised and thermally excited to higher energy levels. No chemical bond can survive the temperatures of the ICP and the "atomisation" of the analyte solution entering the ICP should be complete. Many atoms are converted to the ionised state (singly, doubly charged ions etc being produced). Radiation of characteristic wavelength of particular atoms of an element are emitted in the optical region of the electromagnetic spectrum during transition of the atoms back to the ground state. Radiant energy equivalent to the amount of energy initially absorbed by a particular atom in the excitation process is emitted. The radiation is resolved by an optical filter into spectral lines, from which the chemical elements may be identified and their concentration in the sample determined.

DIFFERENT STAGES OF ICP ANALYSIS

The ICP is used as a mean of emitting light. The light is emitted by different elements at different wavelengths and the intensity of light increases as the concentration of the element increases.

In the ICP analysis, the sample can be introduced into the ICP either in the solid or solution form. In our analysis, a solution method of analysis was adopted. The different stages of analysis are described below:

NEBULIZATION

The first stage of the analysis is the introduction of the sample which is in the solution form into the ICP torch as an aerosol. The sample solution to be analysed is pumped into the nebulizer where it is converted to an aerosol by the flow of an Argon gas. Nebulizers are of different designs but the common ones are the cross-flow type in which two capillary tubes are mounted at right angles in the same plane. Solution is pumped through one and an Argon gas flows through the other. The solution is thus converted to an aerosol (with Argon), the final droplets travel on into the ICP torch and the larger droplets fall and are removed by the drain system.

TORCH AND PLASMA

The aerosol solution from the nebulizer chamber enters the ICP torch itself. The ICP is contained within a two turn coil which carries a high frequency current. When this current flows in the coil, it generates a rapidly varying magnetic field within it. As charge particles (e.g. ionised gas containing our analyte solution) flow through the field, the magnetic lines of force are cut resulting into heating which generates the ICP flame. The inductive heating effect maintains the ICP flame at temperatures of 6000^0 K and up to $10,000^0$ K at its hottest point. The coil is water cooled to remove excess heat. The high frequency current for the coil is provided by an r.f. generator. In our case, the frequency used for the oscillating current is 27.12MHz. However, recent instruments have tended towards higher frequencies by using generators up to 56MHz.

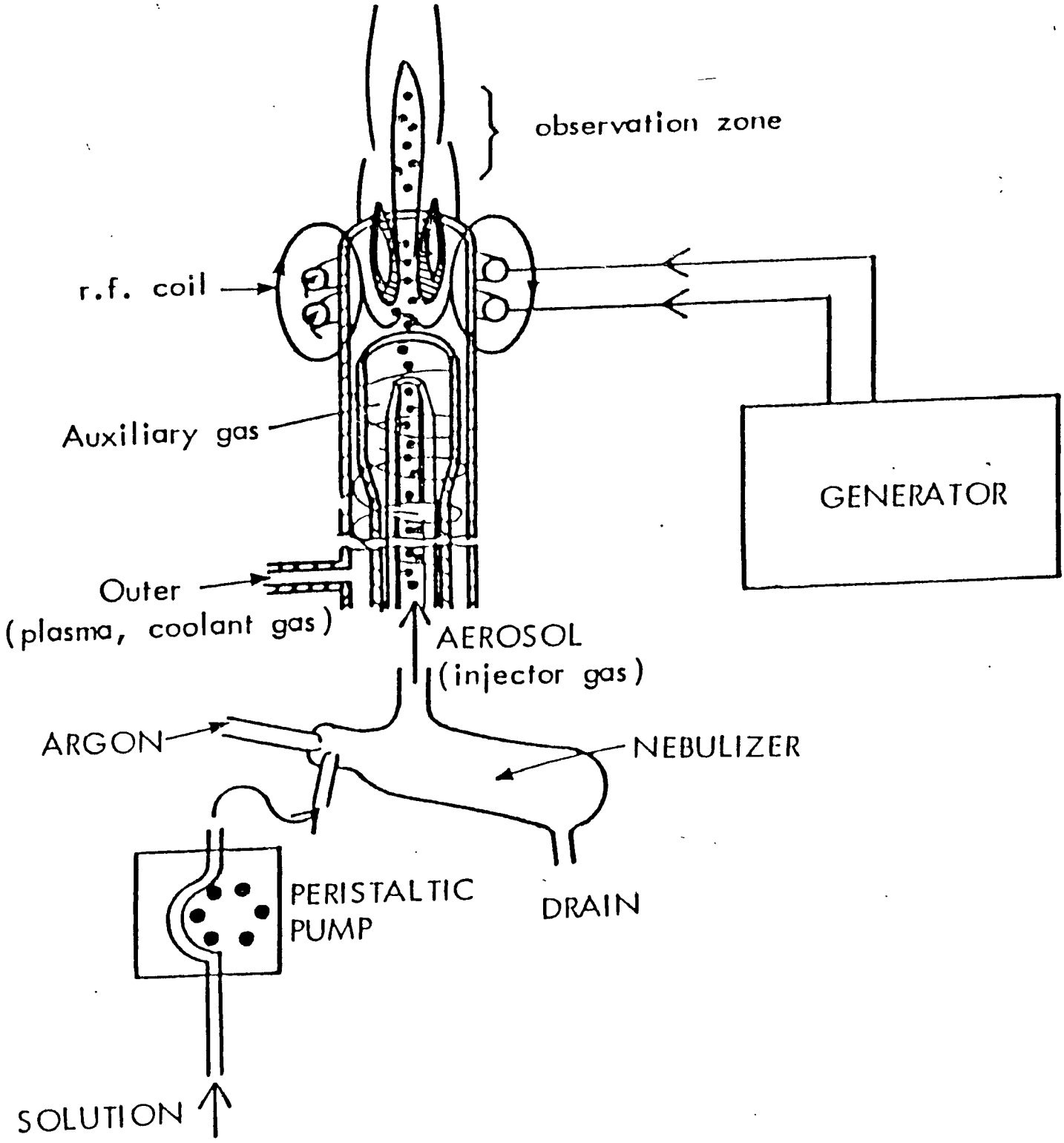


FIG-4. THE GEOMETRY OF ICP TORCH

FIG-4 shows the geometry of the ICP torch used to generate the ICP flame. The plasma is generated in the centre of the tube and the outer part of the gas flow cools the glass tube. The method by which the sample is introduced into the ICP is possibly the most important part of the use of the ICP for quantitative analysis. The plasma is generated in the outer tube, and the sample injected through the flattened base of the flame. Therefore heating of the sample occurs from the outside inwards and the sample in effect enters along a central tunnel.

SEQUENTIAL SPECTROMETERS

FIG-5 shows the schematic layout of a sequential ICP spectrometer system. Here only one channel or monochromator is used. The diffraction grating is rotated under computer control using a stepper motor to locate its position. Thus it is possible, using only one optical system to measure an unrestricted selection of spectral lines.

Practical analysis of the sample using sequential ICP system consists of first entering the wavelength selection programme into the computer. The analyte solution is sprayed into the ICP and when the signal is stabilised, the computer drives the grating to the first wavelength line. This is then repeated for all programme lines and the computer then calculate results.

SIMULTANEOUS SPECTROMETER

A multichannel spectrometer or polychromator is used for simultaneous determination of the elements. The light emitted by the ICP source is focussed into the spectrometer. The diffracting grating resolves the light into its component wavelengths and this then falls on a series of light sensitive photomultipliers tubes located at precise wavelength in the diffracted light spectrum. In the polychromator system, only those wavelengths installed in the spectrometer can be measured. Each elements has a selection of lines that might be used for analysis. Most high quality ICP polychromators now use 1 to 1.5 meter spectrometers which offer a practical resolution of better than 0.03nm. **FIG-6** shows the schematic diagram of a simultaneous ICP system.

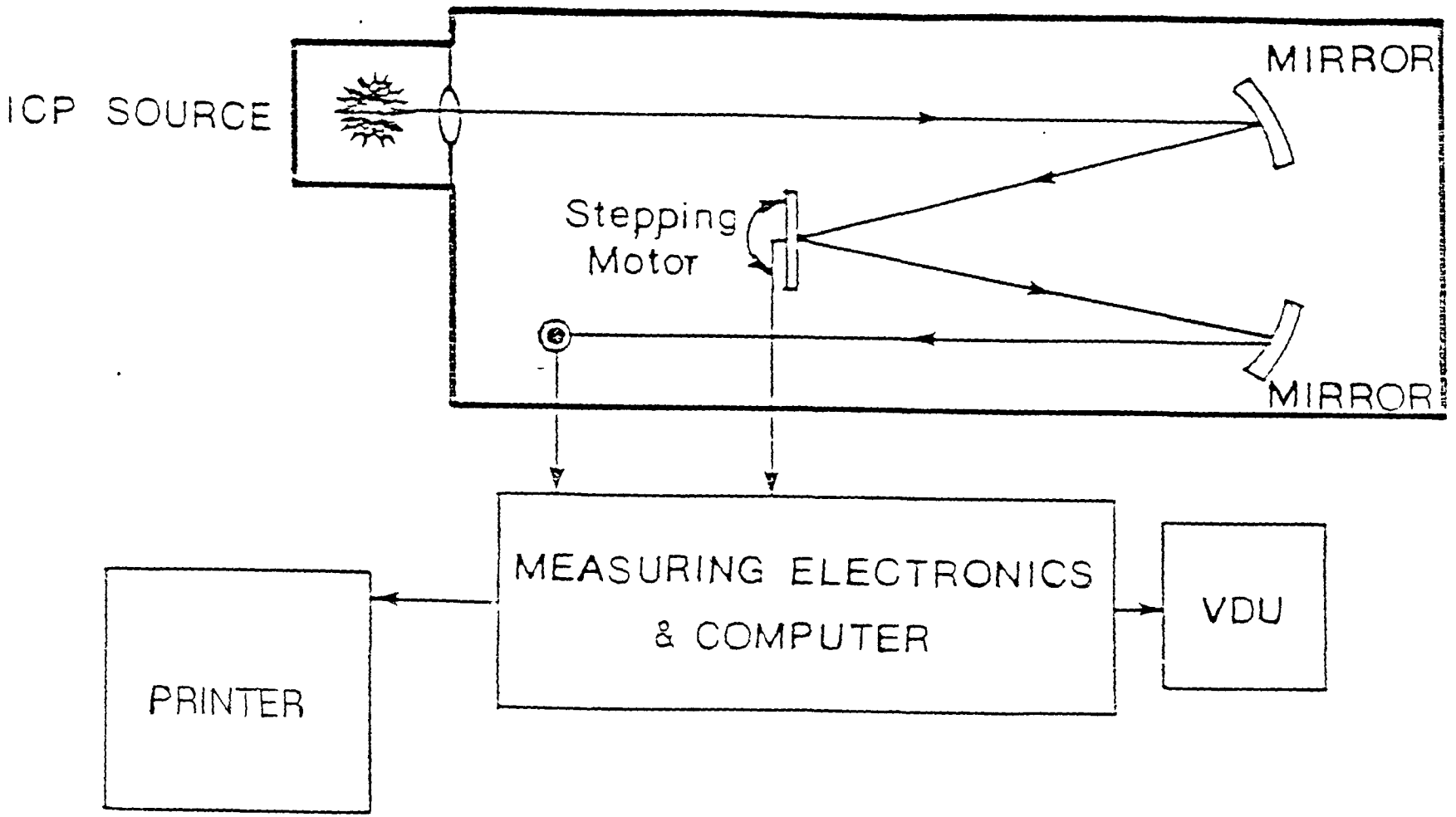


FIG-5. SCHEMATIC DIAGRAM OF SEQUENTIAL ICP SYSTEM

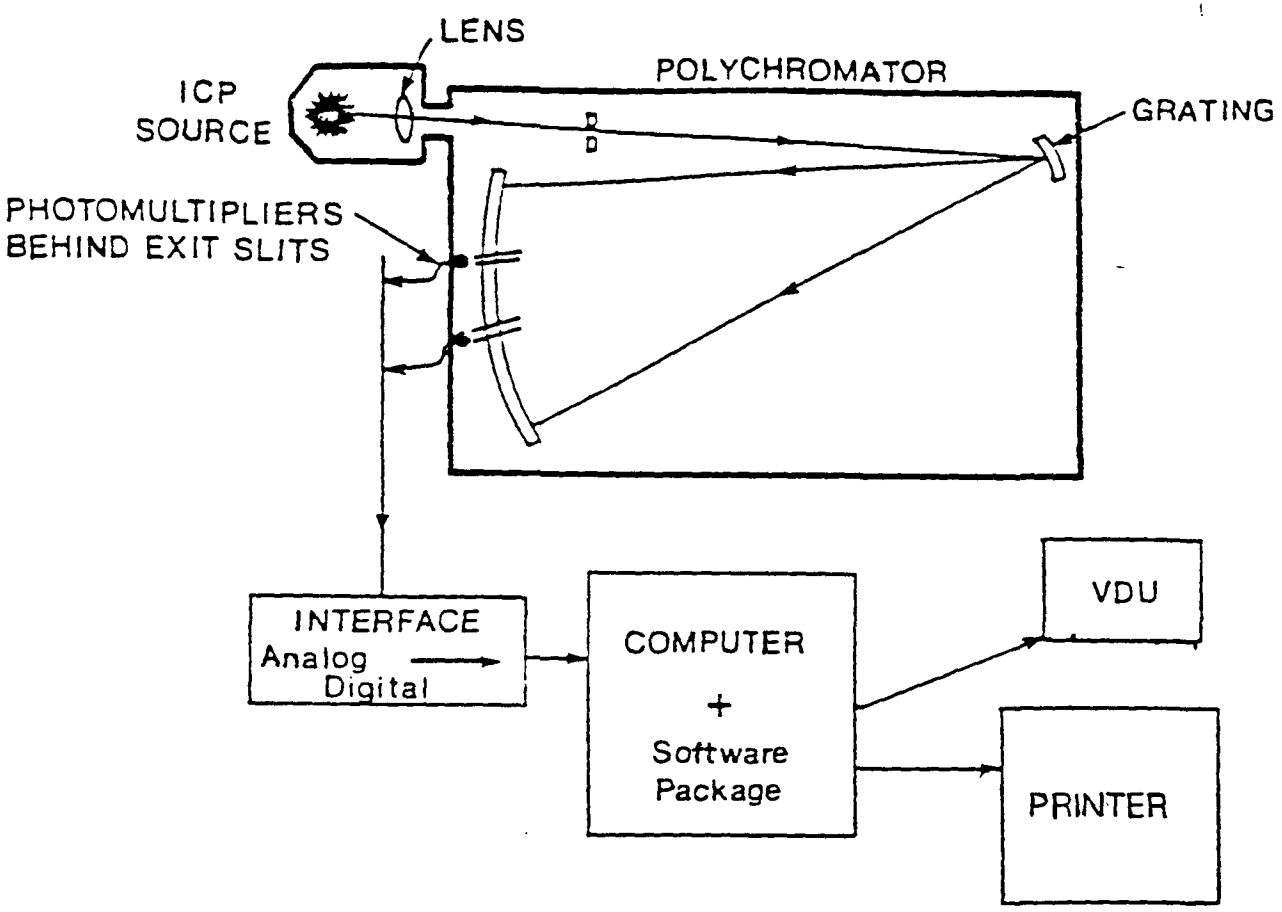


FIG-6.. SCHEMATIC DIAGRAM OF SIMULTANEOUS ICP SYSTEM

CALIBRATION

To calibrate the instrument, appropriate emission lines for the element of interest in the sample or matrix were selected. In case of sequential ICP system only one PMT is used for all the elements. This system was used throughout our analysis. In the case of a polychromator, one PMT for each element spectral line is mounted at the required position behind a narrow slit assembly. A series of standard solutions was prepared covering a range of concentrations anticipated for the element to be measured. The standard solutions were run on the ICP system. The light signals produced in the ICP, generated a mV signal in the PMT. This electrical signal was fed to the capacitor and then to a digital voltmeter. A dedicated computer system attached to the instrument was calibrated with the appropriate standard solutions and read out results directly as concentrations. The software packages **"SLIMPAC" version P.1** was used to have calibration plots of concentration against signal output. A straight line relationship between the signal and the concentration of the element being measured was then obtained.

The signal level for each element was stored (as a calibration line) in the computer system. Two points on each calibration line (a high point and low point) were used as "recalibration" standards on a day to day basis. Thus after recalibration was performed to allow for instrumental drifts, unknown solution was run and concentration equivalents for the signals obtained calculated. The day-to-day recalibration of the ICP system was shown in **FIG-7**.

The calibration curve of ICP offers a wide dynamic range. This has been one of the critical attributes in establishing the technique as a realistic multi-element method. The calibration lines that were stored in the computer for routine analysis could cover concentration in solution of less than 1µg/litre (ppb) upto over 1000 µg/ml (ppm) and remained initially linear over the whole range. Calibration lines did not show significant line curvature below 1000µg/ml, unless the instrument was incorrectly set up. The calibration procedure is crucial to the effective use of the spectrometer system for quantitative analysis.

Thus after initial peak finding of the first standard, the blank solution was aspirated as a second standard in order to get a calibration curve.

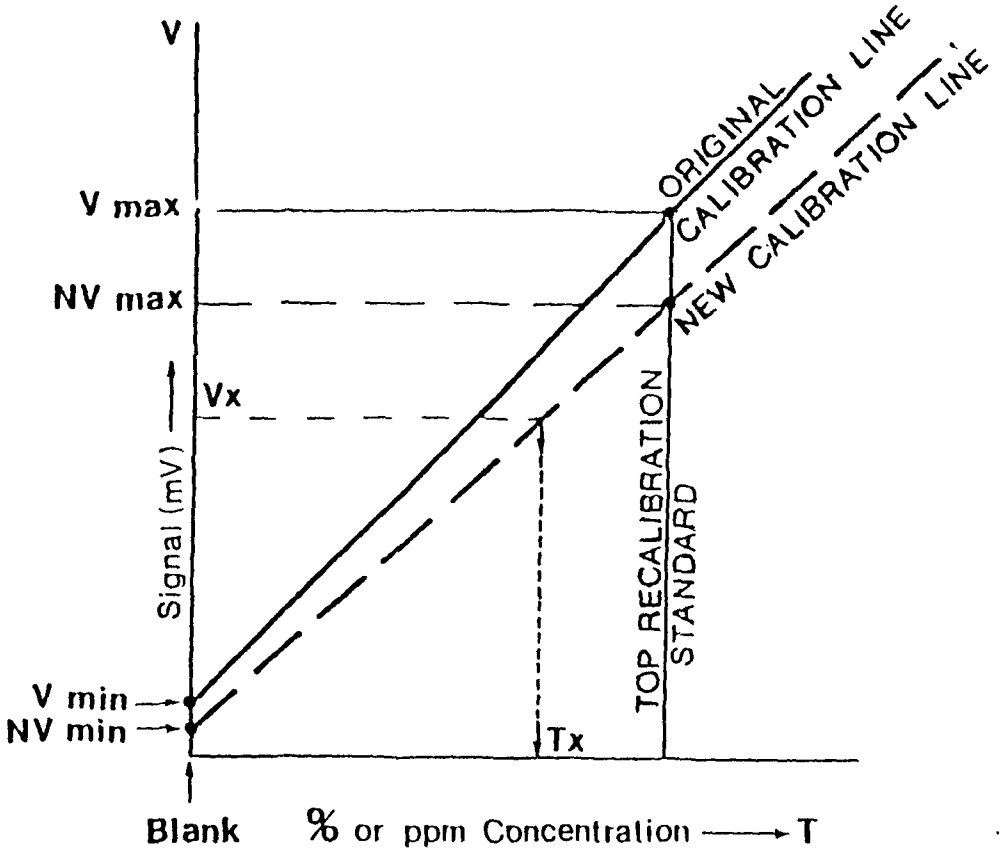


FIG-7. RECALIBRATION CURVE OF THE ICP SYSTEM

Calibration lines for all elements to be analysed are prepared from plots of mV signal intensity (V) against concentration (T). This plot is entered into the computer, together with the intensity values for two standards (a high point [V_{max} and a low point V_{min}]. When routine analysis commences these two reference points are rerun. Changes in instrumental settings will move them to new mV intensity values NV_{max} and NV_{min}. The calibration line is then "moved" to the new line. Thus it is possible to run an unknown sample X, which gives an intensity reading V_X, and read off directly the concentration result T_x. For multielement analysis high and low points for different elements can be combined into multielement solutions. In theory, only two solutions are required for recalibration, in practice more will usually be necessary.

PREPARATION OF STANDARD SOLUTIONS

In our analysis, standards solutions were acquired from the Aldrich chemical company and were specifically meant for trace elements analysis using ICP only. Different concentrations of standard solutions were prepared by diluting the stock solution with distilled water according to the requirement. Also, standard solutions of some elements which were not available for us were prepared using simple salts of Potassium chromate ($K_2 CrO_4$), Ammonium heptamolybdate tetrahydrate [$(NH_4)_6 Mo_7O_{24}.4H_2O$] for Cr and Mo respectively. by dissolving in water or dilute acids.

SAMPLE PREPARATION METHODOLOGY

The methods of sample preparation for ICP analysis are diverse. We are describing here only the solution method which was adopted throughout our analysis. In many respects, the solution aspect of the method is a great advantage. This method first ensures that all the elements to be determined are in the same form. Sample dissolution methods are capable of considerable improvement and automation. Moreover, solutions are easily stored, and many automatic samplers are available to handle introducing the liquids into the ICP. The great advantage of dealing with a solution method of analysis is the flexibility it gives to the analysis.

In the solution method, the mineral acids such as HCl, HNO_3, $H_2 SO_4$ are widely used for sample dissolution. But for refractory samples like geological samples, Hydrofluoric acid (HF) is often used successfully. The excess HF is usually removed by evaporation.

DETECTION LIMIT

The detection limit is defined as that amount of analyte in solution which would give a net increase in intensity equal to three (3) times the standard deviation of the blank.

The detection limits for ICP on pure solutions are quite outstanding. But the detection limit obtained on pure solutions cannot be transferred directly on to real samples. If this can be done, detection limits of less than 1μg/g (or 1μg/ml) in the sample will be achieved for many elements in most applications. In the case of solid

samples, this cannot be obtained as the dilution effect from dissolving the samples is typically of the ratio 1:100. The Detection Limit of a few elements of interest in our study is given in **APPENDIX-IV**.

OPTIMAL OPERATION PROCEDURES

The parameters that generally have the most effect on the performance of an emission line are viewing height, sample gas flow rate, intermediate gas flow rate, peristaltic pump speed, and forward power, although any operating parameters may be used in any combinations.

1. **Viewing height of the torch:-** The height of the torch should be viewed properly so that it sits 15mm from the top of the working coil and also it should be centrally located within the diameter of the working coil. This height can be adjusted by grasping the torch and moving it up and down within the friction mount.
2 **Sample gas:-**One of the parameters to be considered which has a significant effect on the performance of the instrument is the gas flow rate. To form a stable plasma, a pattern of three argon gas flows is used as shown in **FIG-4.** The innermost gas called the sample gas or the carrier gas, transports the sample to the plasma either as an aerosal, a vapour or a thermally generated vapour and the sample gets atomised. In the case of our Instrument use, the sample was transported in the form of aerosol. The rate of argon flow is approximately 0.5 to 1.0 L/min. The sample gas pressure is set at 400Kpa which corresponds to a flow rate of approximately 0.9 L/min of argon to the plasma. The gas control panels were not changed when the instrument was set. The alteration was made only when a new matrix was desired in order to have optimising conditions.
3. **Intermediate gas:-** The intermediate gas known as Auxiliary tube gas or middle tube gas, helps in stabilising the base of the plasma. The argon gas flow rate in our case is set at 1 to 2 L/min and the normal operating pressure was set at 100 to 200 Kpa. The flow rate of this gas is also regulated by rotameter needle valve located on the front panel of the instrument.

4. **Peristaltic pump speed:-** The speed of the peristaltic pump which takes the sample to the nebulizer is an important factor to be considered for getting good precision. The function of the pump is to control the flow rate of the analytical solution and to eliminate the viscosity. For our work, the speed of the pump was so adjusted so that the uptake was maintained between 1.8 to 2m/min. The working life of the pump tubing depends greatly on the speed of the peristaltic pump and normally it is about 40 hours. Once the pump tubing loses its elasticity or feels flat or lumpy, it should be replaced immediately otherwise the precision is worsened.
5. **Forward power:-** The forward power is being fed into the plasma by a Radiofrequency generator. There is a 10 turn potentiometer marked on the forward power preset which can be used to set the forward power to the desired analytical conditions. For our work, the forward power is set at 1.2KW since our samples were in the form of aqueous solutions. If organic solutions are used, the power is to be set at 1.7KW for the similar system and the frequency for the oscillating current is maintained at 27MHz.
6. **Wavelength selection:-** Wavelength selection is very important in analysing the samples. In sequential mode (monochromator), ICP spectrometer can select any required spectral line in an analytical programme. This is useful when the sample matrix changes from one analytical requirement to the other. The monochromator wavelength ranges from 160-820nm. In simultaneous (polychromator) mode of analysis, wavelength selection is very important and depends on the matrix of the samples and their concentration range. Samples containing many elements to be analysed often emit a complex series of spectral lines. Therefore, proper selection of wavelength has to be done and failure to resolve these lines will completely lead to spectral overlaps and interelement interferences. The line is chosen so that there is no strong interference from the matrix.

PROBLEMS ENCOUNTERED DURING OPERATION

1. **Plasma ignition difficulties:** The high tension ignitor lead is to be checked whether it is properly connected to the torch or not if the argon gas cannot be ionised even when

there is a forward power and tuning of reflected power has no problem. The improper flow of argon gas to the torch also contributes the above difficulty

2. **Computer problem:** The computer is interfaced with the instrument for analysing the data. While using this instrument for our work the problem faced by us was that the computer after successfully booting, stops to address the data. This problem was overcome by first checking the interconnecting cables and then resetting it. The other problems like boot failure or no activity on the screen were not seen by us though there are remedies to overcome them.
3. **Torch height:** The height of the torch in the working coil should be adjusted properly so that it is low but not too low otherwise the plasma cannot be started. Even if it starts, it goes out very easily. Moreover, proper alignment is desired and the concentricity of the torch should sit in the middle of the working coil. The typical distance of the torch from the bottom of the work coil to the top of the intermediate tube is 1 to 1.5mm for aqueous samples and 1 to 2.5mm for organic samples.
4. **Gas control:** The gas lines are to be checked carefully making sure that there is no leakage and possible contamination. The flow rates and pressure of the three argon channelled argon gases namely sample gas, auxiliary gas and coolant gas should be set to the values prescribed by the manufacturer. A difference of + 2% from the given values is tolerable in order to have good analytical precision.
5. **Calibration:-** A number of standards and a blank are used for calibration. The standard solution is aspirated first in order to find the actual peak maxima for each line and locate it accurately. After the initial peak finding of the first standard, the blank is run. One has to be very precise in locating the actual peak maxima otherwise the computer will not accept it. Also the matrix used to prepare the calibration standard solution should not ideally contain any detectable amount of the elements to be calibrated. For e.g. if the calibration standards for a particular application are prepared using 5% HNO_3 solution, the standard blank must also be used of the same concentration HNO_3 solution. In our analysis, standard solution are used supplied by the Aldrich Chemical Company. The working solutions are prepared

by diluting from the higher concentration stock solutions supplied by the company. Diluting to the required lower concentration were made accordingly.

INTERFERENCES

One of the most important factors in the application of an analytical technique is the degree of feedom from interelement interaction or interferences(matrix effects). This interference by concomitants (constituents in the sample other than the analyte) may be spectral or nonspectral in nature. But ICP is an analytical technique less affected by interferences than most alternatives.

1. ***Acidity of analyte solution:- It occurs when the signal emitted by an element is influenced by the level of mineral acid in solution as different mineral acids have greater or lesser effects on the emission signal. This can be overcome when the standards and the unknown samples acidities match within about 10%.***
2. ***Suppression of signal by excess of major elements:- This interference supresses the emission signal of the element of interest due to the presence of considerable amount of other elements in the sample solution. The supression may be due to the change of the viscosity of the solution which ultimately affects the performance of the nebulizer. It is usually overcome by sensible matching of standards and sample matrix concentration.***
3. ***Sample transport and excitation effects:- sample transport effects include factors such as changing sample viscosity, surface tension and vapour pressure. It may be due to the presence of easily ionisable elements, changes in sample matrix, or recombination spectra. All these effects can be mostly dealt with by using matrix matching techniques for both standards and unknown samples.***
4. ***Optical Spectral interferences:- While analysing the samples, the complex spectra emitted by the elements can never be completely resolved and the overlapping of the spectral lines of different elements always occur to some extent. This kind of interferences depends on the magnitude of the overlap and relative concentration of the analyte and interferences. There are three types of spectral interferences and these effects can be attributed as follows:***

(a). **Direct spectral overlap**:- It occurs with the interfering line lying directly underneath the analysis line. This is overcome by improving the spectral resolution and mathematical correction is made provided the magnitude of interference is not too great.

(b). **"Wing" overlap** :- This effect is similar to that of direct spectral overlap. Here the interference is from a line which is not immediately close to the analysing line but which is further away. This partial line overlap is improved or avoided by increasing the resolution of the spectrometer.

(c). **Increased background signal:-** This interference takes place beneath an analysis peak which occurs on both sides of the peak. It can be overcome by measuring the background on either side of the peak. This typical interference is found in the case of Aluminium which emits a background continuum in the region of the spectrum 190-220nm. The ICP which we are using is fitted with a movable entrance slits to the spectrometer, thus allowing this correction to be made automatically during routine analysis.

SPECIAL CHARACTERISTICS OF THE TECHNIQUE

1. The high sensitivity and the possible simultaneous excitation of as many as 72 elements notably metals and metalloids, have made this technique suited for rapid survey analysis of the elemental content in small samples at the level of 10 μg/g or less. Generally less than 5ml of the sample solution is required for the analysis.
2. The ICP discharge provides a rich spectrum for qualitative and sequential or simultaneous multielement quantitative analysis with good accuracy.
3. Another advantage of the plasma excitation is the virtual elimination of the molecular bands attributable to the metal oxides which form refractory oxides in air-fuel flames.
4. Gases and solution can also be handled with ease while soild samples can also be used.
5. The operating cost of these plasmas, exclusive of electrical power, is lower than the cost of the gases needed to operate the Nitrous Oxide-Acetylene flame commonly used in AAS.

6 ICP is almost freed from chemical interferences due to its high temperatures of the plasma which is 10,000° K.

7. Analytical linearity of the ICP far exceeds that of AAS.

8. The high degree of freedom from interelement effects makes it possible to establish a single set of calibration curves for the determination of analytes in a variety of sample matrices.

9. Multiple analysis can be performed on a few milligram amounts of sample. Hence many elements can be determined readily by this technique which is difficult to analyse by conventional procedures.

10. For qualitative analysis and identification of trace impurities, emission spectrometry is undoubtly superior to most other techniques. The accuracy obtained for quantitative determination of trace amounts of materials is not inferior to other techniques.

11. The range of application of emmision spectrometry is extremely broad. The technique has been used successfully for the analysis of rocks, minerals, metal alloys and commercial products of all types.

The ICP is a powerful technique in the detection of several elements. However there are some limitations and a few of them are listed below:-

1. Using this technique for the analysis, it requires the accurate selection of the wavelengths of the various lines.

2. The total cost of the instrument is expensive. The approximate cost of our instruments is 32 lakhs.

3. The time taken for one analysis is more when compared with other techniques like AAS. This is because it takes a much longer time for a signal to reach a steady level after the begining of sampling. Also, the integration time required by the ICP to provide a given degree of precision is longer.

4. ICP nebulization systems are prone to clogging. Only 1-2% of the solution entering finally reaches the ICP, the remainder drains away to waste while in Atomic Absorption systems, 10% of the solution may reach the flame.

5. Under experimental conditions commonly employed, the plasma is observed in an air environment. Thus the determination of O_2 and N_2 is usually not possible. Also the determination of Br, Cl and F is not practical because their useful lines fall in the UV region. But at present, this problem can be solved by the inclusion of other facility requirements.

2.3 X-RAY FLUORESCENCE

INTRODUCTION

The detection and estimation of elements present in the samples by x-ray fluorescence (XRF) involves the use of x-rays as the projectile. These rays were first observed by William Rontgen, a German Physicist. In 1895 he found that when a beam of high energy electrons was allowed to strike the metal target in an evacuated tube, some of the kinetic energy was converted into a very penetrating form of electromagnetic radiation. As he could not understand their origin, he called them "x-rays". Twenty years later, Max Von Laue, another German physicist discovered that these x-rays are diffracted by atoms in a crystal, similar to the diffraction of the ordinary light by a line grating. Shortly thereafter, Henry Moseley, an English physicist, studied x-rays emitted by a number of elements and found that the wavelengths are characteristic of the element. Few years later, XRF became an important tool for trace elements analysis. It offers a rapid, non-destructive multi-elemental analysis of samples having concentration levels as low as few parts per million (ppm). A unique feature of this technique is that it permits the simultaneous determination of all elements ($Z > 11$) in a single run. It is rather an inexpensive technique and very simple to use. The technique has been improved and extensively used by several workers [11-13, 27-33].

PRINCIPLE OF X-RAY FLUORESCENCE

When an atom in a specimen is bombarded by high energy photons (x-rays or γ-rays) or high energy charged particles, the photon or the particle collides with an atomic electron giving part of its energy to the electron and making it get excited (if $E < B.E.$).

Within a very short time it comes back to its original state emitting the energy difference between the levels in the form of electromagnetic radiation. If the energy with which it interacts is greater than the binding energy of the electron (E > B.E.) it can eject the electron out, thus ionising the atom. The vacancy created in the atomic structure is immediately filled up by another electron from the upper levels in less than 10^{-15} secs. The energy difference between these two levels is emitted in the form of characteristic x-rays. This x-ray is used to identify the atom from which it comes and its intensity determines the concentration of number of atoms and hence the element. **FIG-8** shows a simplified picture of fluorescence process to generate characteristic X-rays. **FIG-9** shows a typical partial energy diagram illustrating the transitions leading to K and L series of X-rays.

The equation governing the analysis is given as

$$I_j = I_0 \; G \, K_j \; M_j \; A \qquad \ldots\ldots\ldots\ldots\ldots\ldots \qquad (1)$$

Where,

I_j = Intensity of the characteristic x-ray of the jth element of a given sample

I_0 = The incident photon flux on the sample

G = the geometrical factor = $G_1 G_2$

Where G_1 is the geometrical factor between the source and the sample and G_2 is the geometrical factor between the detector and the sample. For a given set of sample, the source and the detector G is a constant factor.

K_j = the relative ability to excite and detect the x-ray line of interest.

M_j = concentration of the j^{th} element (gm/cm^2)

A = Total absorption correction factor which is due to absorption of exciting and fluorescent radiation in the sample matrix.

Considering the sample to be thin, the matrix enhancement effect is neglected. This occurs when the fluorescent x-rays of one element act as an exciting radiation for other elements whose absorption edges is lower than the energy of the fluorescent radiation of the element. The effect is severe for thick samples and it is difficult to correct without prior knowledge of the specimen.

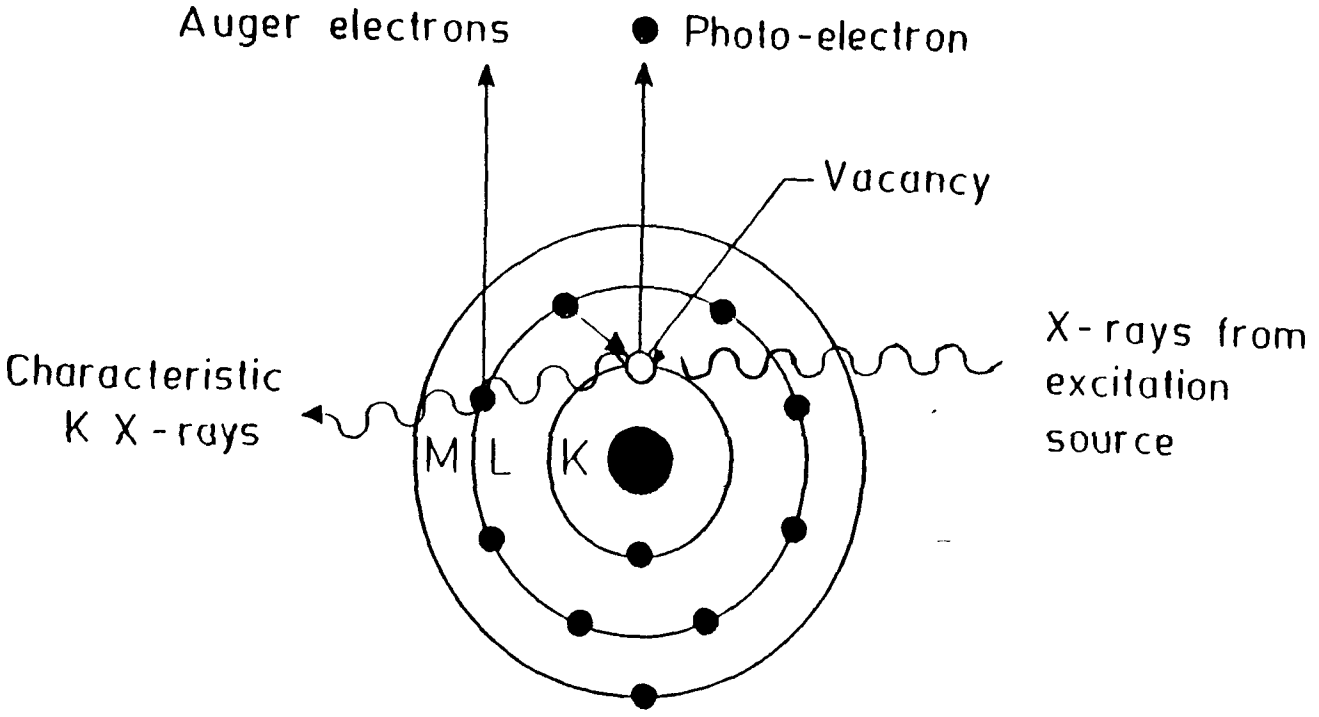


FIG-8. SCHEMATIC DIAGAM SHOWING THE GENERATION OF CHARACTERISTIC K X-RAYS FROM SODIUM ATOM

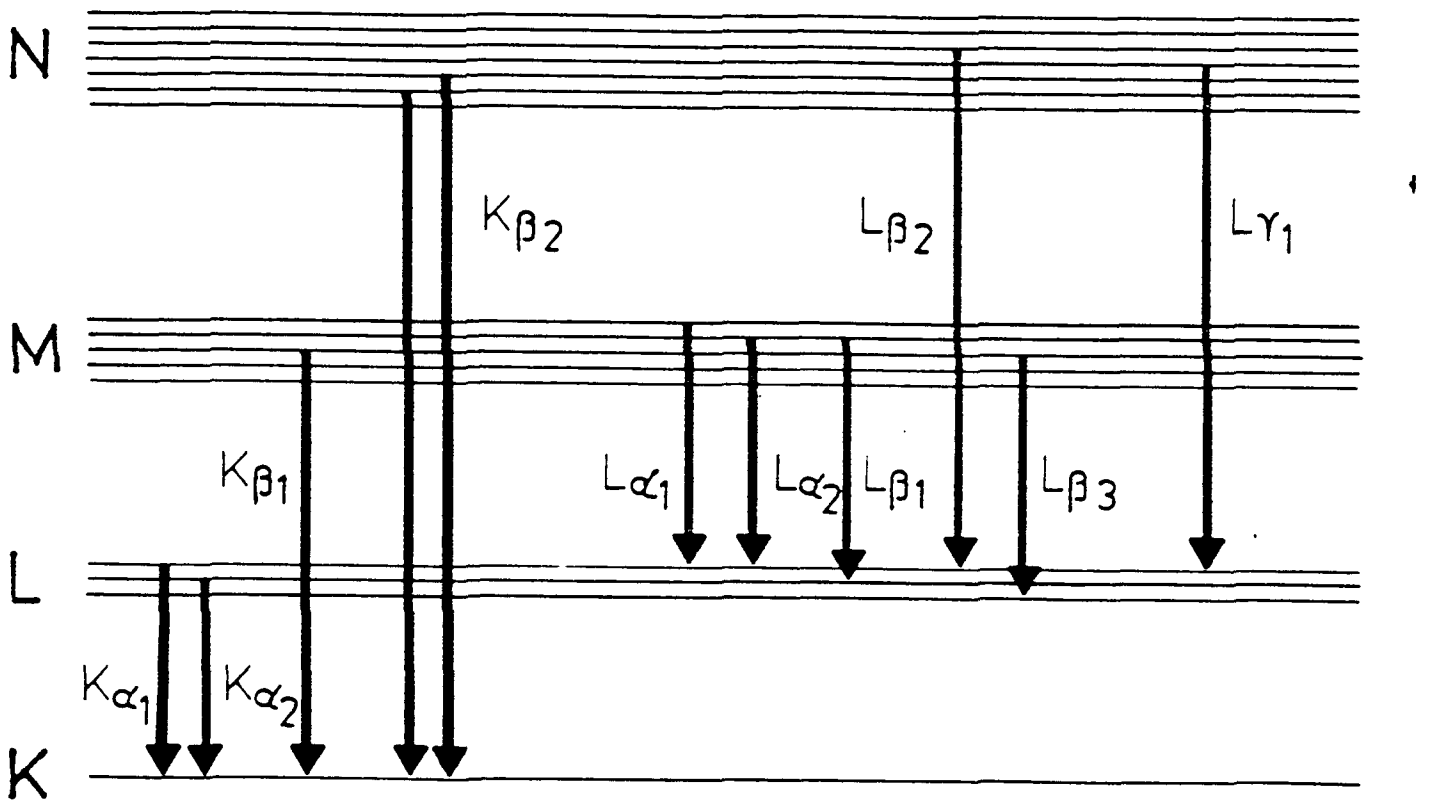


FIG-9. PARTIAL ENERGY LEVEL DIAGRAM SHOWING THE TRANSITIONS LEADING TO THE K AND L SERIES OF X-RAYS

The total absorption "A" is obtained by considering the absorption of a small element thickness in the specimen and then integrating over the entire specimen thickness.

$$A = \frac{1 - e^{-(\mu_1 \operatorname{cosec}\phi_1 + \mu_2 \operatorname{cosec}\phi_2)m}}{(\mu_1 \operatorname{cosec}\phi_1 + \mu_2 \operatorname{cosec}\phi_2)m} \quad \ldots\ldots\ldots\ldots (2)$$

Where,

ϕ_1 and ϕ_2 = are the average angles of incident and fluorescent radiation with respect to the sample surface [FIG-10]

m = mass of the specimen per unit area for the geometry choosen.

μ_1 and μ_2 = the total absorption coefficients of the specimen for the exciting and fluorescent radiation respectively.

To determine μ_1 and μ_2, the sample is diluted by mixing it with a large amount of low Z material such as pure cellulose powder, borax etc. Though diluting the samples would results in lower analytical sensitivity the matrix enhancement effects of the diluted sample are insignificant. Only simple matrix absorption corrections are required by using equation (2). For thin, diluted samples, the compton scattered peak of the primary monoenergetic beam is mainly determined by the cellulose matrix. The ratio of the intensity fluorescent peak to the intensity of the compton scattered incident beam depends on the concentration of the respective element with respect to the cellulose. Therefore, the system can be easily calibrated using a set of multielement standards prepared in the above mentioned manner.

The relative ability K_j is given by

$$K_j = \sigma_{kj}\, \omega_{k,l} \left(1 - \frac{1}{J_{k,l}}\right) T f E \quad \ldots\ldots\ldots\ldots (3)$$

σ_{kj} = the photoelectric cross section of the element j and of the atom in the K shell.

$\omega_{k,l}$ = the fluorescent yield

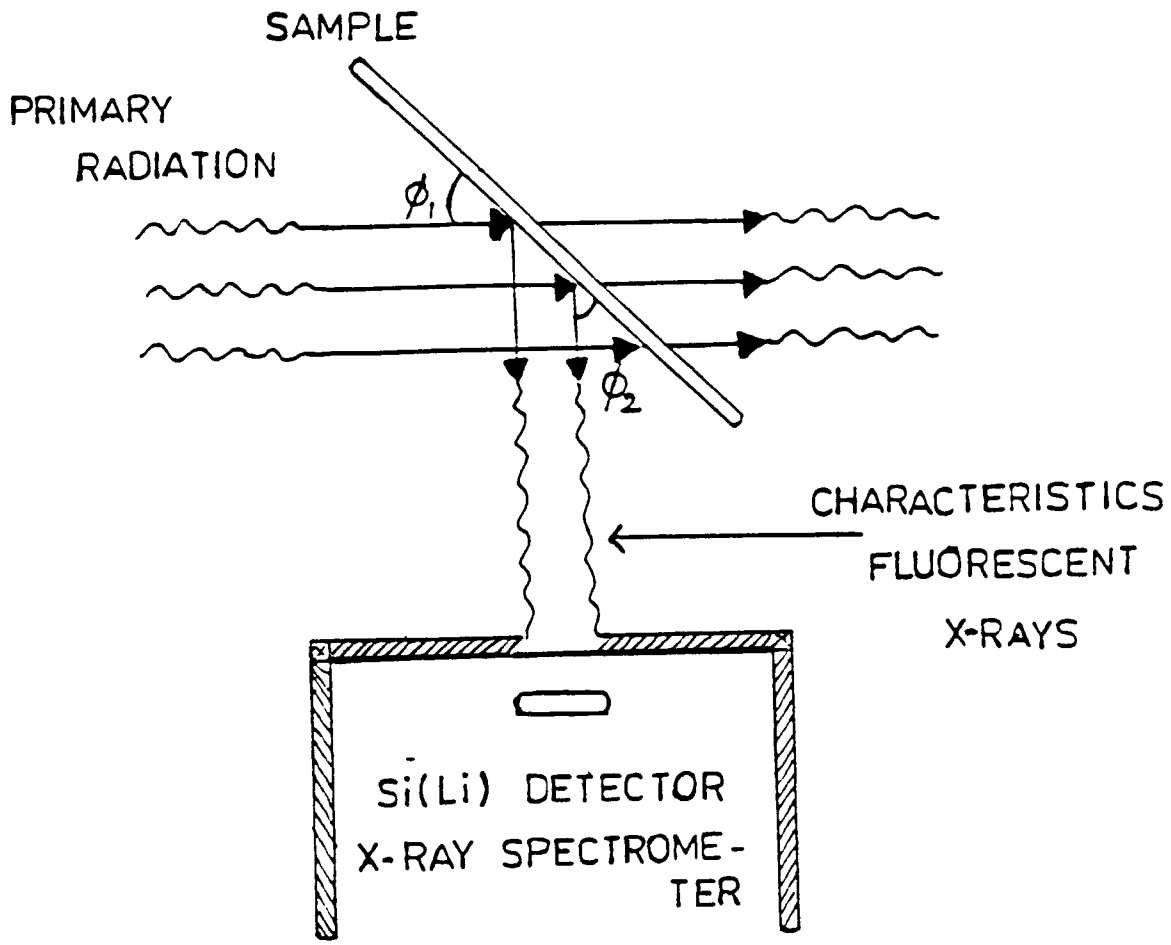


FIG-10. SCHEMATIC DIAGRAM OF X-RAY FLUORESCENCE

$J_{k,l}$ = the jump ratio for the element j

T = the transmission of the fluorescent radiation in its path.

f = the fractional intensity of the x-ray line under analysis.

E = the detector efficiency.

The values of $\omega_{k,l}$, $J_{k,l}$, σ_{kj} and f are obtained from the literature and with the known values of T, E for the detector, the factor K_j can be evaluated for a given system. The value I_0 G can be determined by adopting one element standard method. In this method, one non-interfering element of known concentration is either added to the specimen or taken as a separate specimen. Knowing the intensity of the characteristic x-rays of known elements, the value of I_0 G is computed as given in equation (1).

Putting all the values in equation (1), M_j can be known.

Then M_j in ppm is given by

$$M_j \text{ (ppm)} = [M_j / M] \times 10^6$$

Where M = mass of the sample (g/cm^2).

In some cases, the separation of the K_β line of one element with the K_α line of another has to be done. In other cases, the L-x-rays of the higher elements might overlap with the $K_{\alpha\beta}$ -x-rays of the lower elements. A knowledge of the intensity ratio of all the x-ray components is required and applied to resolve them.

EXPERIMENTAL SET UP:

It consists of an excitation source, a sample holder, a Si(Li) detector cooled in liquid nitrogen, a charge sensitive preamplifier, a main amplifier and a PC based Multi-channel analyser (MCA) system. The measurements were carried out in a coaxial geometrical arrangement of the source-sample-detector. The block diagram of the set up is given in **FIG-11**

SOURCE : Source that can be used include x-ray tubes and the high energy photons (x-ray or γ-rays). Sometimes, high velocity charges particles are used. The excitation source

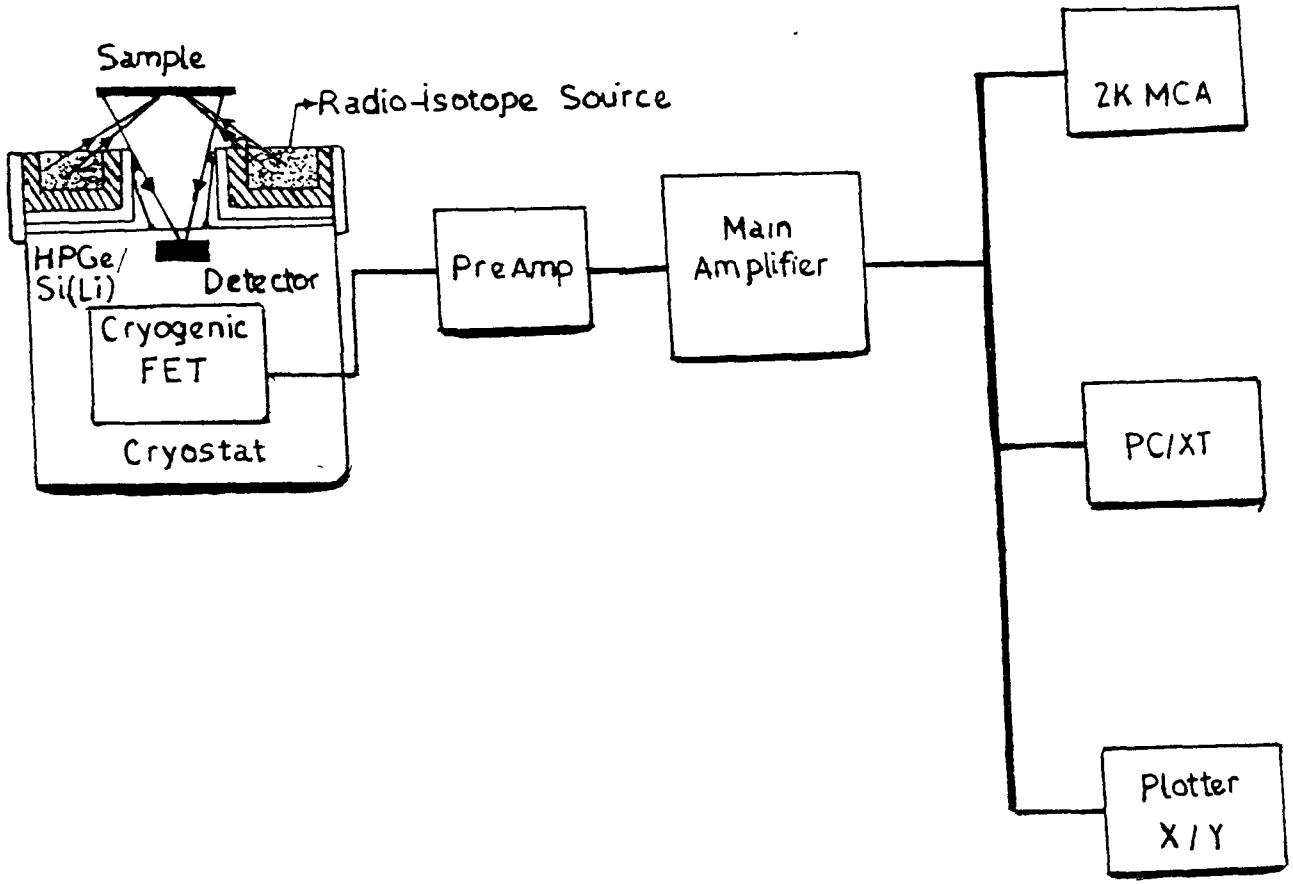


FIG-11. SCHEMATIC DIAGRAM OF THE EDXRF SYSTEM

used for our analysis was a radionuclide ^{109}Cd source (activity of 740 MBq, Ag K-x-rays-22.2 Kev; Half life(T) = 1.3yr) of 30mc strength in annular geometry mounted on the end cap and shielded from the Si(Li) detector. It has the capability of high sensitivities for excitation of elements from Gallium to Niobium.

SAMPLE HOLDER: It is placed in a precisely defined position during analysis and provided for introduction and removal of the sample from the excitation position.

DETECTOR : Si(Li) and Ge(Li) detectors are widely used for detection of x-rays owing to their rapid multi-element analysis capability, excellent resolution and high efficiency. The Si(Li) detector is prefered for lighter elements (less than 30 Kev). The efficiency is greater than 95% for energies below 20 Kev. On the other hand, Ge(Li) detector have advantages for heavier elements greater than 30 Kev. For our work, we used a Si(Li) detector, cooled to liquid nitrogen temperature and mounted inside an evacuated cryostat with a pulsed opto-feedback preamplifier. The Si(Li) detector employed has an area of $30mm^2$ and thickness 0f 3mm and this detector system gave an energy resolution of about 200ev for 5.9 Kev Mn K_α -x-rays. This resolution is maintained upto a count rate of 20,000 cps, though the useful count rate was limited to only about 12, 000 cps owing to large pile-up rejections at higher count rate. The shift in the peak position as a function of count rate was negligible. In the set up, the main amplifier (x-ray pulse processor) had an option of inhibiting the main amplifier action to avoid overloading due to large amplitude pulses along with the pile up rejection. In the amplifier a Gaussian shaping network was used with a peak width of 15μsec. The pulses from the amplifier were then fed to a 2048-channel analyser (Canberra series 80).

PRE-AMPLIFIER : The pre-amplifier is normally used to amplify the weak signal coming out of the detector as well as the impedance matching. It basically integrates the total charge of the pulse (i.e. a current integrater) and converts it to a voltage signal. The output pulse generated gives the information about the energy of the incident radiation.

AMPLIFIER : The output of the pre-amplifier is fed to the input of the amplifier. The function of the main amplifier is to amplify the signal and in shaping the signal to reduce

pile up. A good amplifier should have the properties like (a) linear amplification (b) low noise (c) stable gain and good thermal stability.

MULTI-CHANNEL ANALYSER (MCA) : After amplifying, the pulse are fed to a MCA. It sorts out the pulses according to their amplitude (pulse-height) and it contains energy information. It assigns a memory location for the pulse height. Every time a pulse comes, the count in the memory address i.e., the channel number is incremented by one. Thus the channel number gives the energy of the incident radiation and the counts give the information about the intensity. A spectrum of counts vs channel number is obtained. The peaks in the spectrum identify the characteristic x-ray and hence the elements present, whereas the area under the peaks gives the concentration of the element. It also has the provision for smoothing and expanding the peaks.

COMPUTER : The PCA (personal computer analysis) coupled to MCA is used to analyse the peak height and peak area. The results analysed were then printed out.

CALIBRATION OF THE SYSTEM

To calibrate the system, a sample having known elements is excited by ^{109}Cd or ^{241}Am. The peaks observed in the spectrum correspond to the elements in the sample whose characteristic x-ray energies are known. If the elements chosen for calibration are such that their characteristic x-ray energies cover an appreciable range(~ 30Kev), then the observed peak energies do not deviate much from the actual x-ray energies over a broad range. Usually, three peaks are used to calibrate the system. Fe K_α (6.4Kev), Pb K_α (10.55Kev) and Sn K_α (25.27Kev) were used to calibrate the system here. A typical calibration curve is shown in **FIG-12**.

TRANSMISSION MEASUREMENTS

Any empirically fitted expression involving relevant parameters can be used to evaluate the mass absorption coefficients of different elements observed in the spectrum. Errors in the parameters which are calculated for some range of energies will be reflected in the calculation of final elemental concentrations. The absorption of the X-rays in the sample are experimentally measured by a transmission measurement. In this

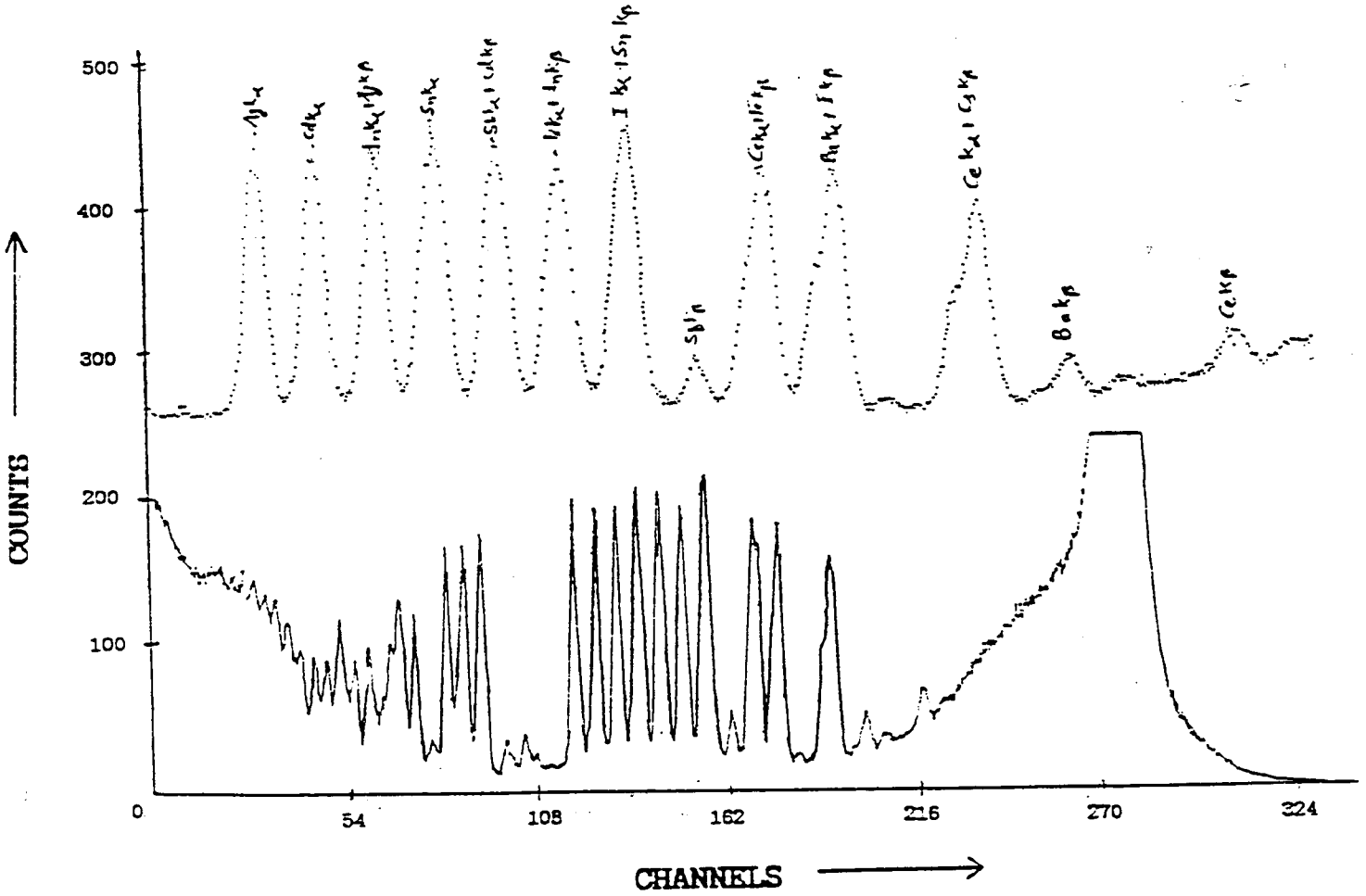


FIG-12. ^{109}Cd PHOTON SPECTRUM OF COUNTS VS CHANNEL NUMBER OBTAINED WITH Si(Li) DETECTOR.

measurement, the sample is inserted between the excitation source and the detector. X-rays from excitation of another foil are transmitted through the sample. The sample is removed and the peak areas are noted for the same time. The relative intensities give a measure of the absorption for different energies. These values are used in the Lagrange's interpolation to find the absorption correction for unknown energies. For standard pellet, the transmission factors have been determined in a similar way and these values are used in the code to compute the elemental concentrations.

MINIMUM DETECTION LIMIT (MDL) : It has been defined in several ways but the definition given by Birks (49) is probably the most widely used. It is defined as the amount of the analyte that gives a net line intensity equal to three times the square root of the background intensity for a specific counting time (usually 100 seconds) or in statistical terms, it is that amount of the analyte that gives a net intensity equal to three times the standard counting error of the background intensity. For a 95% confidence interval it is expressed as:

$$2(2\sigma_B)^{1/2} = 2\sigma, \quad 3\sigma_B = 3(I_B)^{1/2} \qquad \text{.....} \qquad (4)$$

The "concentration at the detection limit" (C_{DL}) is calculated by assuming the counting time to be $T_{peak} = T_{background} = 50$ seconds (100 seconds total time) and entering the slope (m) which is counts/second per 1% analyte which is substituted in equation (4). The value of the C_{DL} is given by the following equation.

$$C_{DL} = [3/m]\ [I_B/B]^{1/2} \qquad \text{.....} \qquad (5)$$

From equation (2), C_{DL} decreases as the background intensity decreases as well as with the increasing slope and longer counting times.

SENSITIVITY, ACCURACY AND PRECISION

The sensitivity, accuracy and precision are based on careful measurements and correction procedures. The main effects which influence analysis are absorption, particle size and enhancement. The first two have a large effect in soil analysis while the

enhancement effect is very significant only in the determination of minor elements such as Fe and Mn. The absorption effect which is due to absorption of exciting and fluorescence radiation in the sample matrix was discussed earlier and it is severe for thick samples. The particle size effects always limit accuracy, particularly when analysing for low Z elements. The question of sample homogeneity often encountered in the analysis is closely related to the particle size problem. This affect is discussed in detail by Berry et al, Hunter and Rhodes and Hunter [34].

Enhancement occurs when photoelectric absorption of a characteristic line by a matrix element takes place. This is more prominent where radiation from a characteristic line is of slightly higher energy than the element being measured and has the effect of increasing the radiation emitted by this element.

The sources of error which are likely to mar the accuracy and precision of quantitative analysis are:-

Counting errors: This becomes large at very high count rates due to dead time and pulse drift.

Instrumental errors: These are generally caused by generator instability, pulse drift encounters and crystal instability with temperature changes.

Variation in operating conditions: Care must be taken to reproduce operating conditions exactly when parameters are changed. Careful sample loading and labelling is also essential.

Variations in samples: Errors due to variations in samples are probably amongst the most important in quantitative analysis. The most important factors are homogenity, particle size, flatness and thickness.

Line interferences: These cause errors which can increase the intensity of the line being analysed or of background determinations on either side of the peak. For example, the line of Cr K_β can interfere with Mn K_α and Mo K_α with Zn K_α .

Calibration errors: Calibration errors can be minimised by the choice of carefully choosen and prepared standards. For soils, adequately analysed natural standards are few.

COMPARISON BETWEEN RADIOACTIVE ISOTOPES AND X-RAY TUBE EXCITATION SOURCE

1. Radioisotopes sources for XRF are small and independent of any external power. These two advantages can be considered to be the most important as they permit the construction and use of portable instruments in many applications of science such as petroleum products, mining, industry, and other fields. They are 10 to 20 times less costly than x-ray tubes and need no service but depend only on their life time. The shielding, handling and maintenance problems are minimal.
2. The extreme source stability of radioisotopes permits the use of long counting times to acquire statistically acceptable counts (which is required for trace elements analysis, since the intensity of the radioisotopes is 10^{-6} to 10^{-7} less than x-ray tube).
3. A ^{109}Cd source is capable of high sensitivities for excitation of elements from Gallium to Niobium.
4. X-ray fluxes obtainable from an x-ray tube are at least a few orders of magnitude larger compared to radioisotopes sources. Therefore, the sensitivity is much better, resulting in smaller data accumulation times.
5. Using x-ray tube, there is a choice of selecting proper anode/secondary fluorescence which enables one to increase the sensitivity for any particular region of interest.
6. The primary radiation coming from radioisotopes as excitation sources for XRF is of low intensity radiation, (10^{-6} - 10^{-7}) less than the conventional x-ray tubes, which is one of its drawbacks.
7. X-ray tube excitation requires a large stable power supply, which is also very costly. The difference in the cost between radioisotope excitation and x-ray tube excitation can be 5-10 times. The output of primary x-rays from an x-ray tube is dependent not only on the stability of the power system but also on the fluctuation in the line voltage. Thus in many cases, it is necessary to have a stabilised voltage on the power line.

8. Compared to x-ray tube, radioisotope induced EDXRF system can excite and detect high energy, k-series x-ray lines. This minimises variation in intensity due to surface thickness or particle size because the penetration of the primary radiation has been from microns to millimeters. The utilisation of k-lines also eliminates most of the overlap interferences between the multiple L and M lines of high Z materials and the K or L lines of the low Z intermediate Z-elements in a sample. The limit of detection is low when the exciting radiation can just be separated from the characteristic x-ray of the element of interest by the detection system. In multi-element analysis, the energy of the exciting radiation must be above the absorption edge of the highest Z-element K-L series x-rays in order to avoid overlap of the K-series x-rays with the L-series x-rays of the heavier elements in the sample.

ADVANTAGES AND LIMITATIONS OF XRF

The main advantages of XRF technique are:-

1. XRF is a powerful technique and offers a simple and quick simultaneous multielement analysis of the sample.
2. The samples are not destroyed during the analysis so that they can be retained for future use.
3. All elements having $Z > 11$ can be detected.
4. A large number of elements both for qualitative and semi- qantitative scan of the element present in the sample can be analysed simultaneously in one experimental run.
5. The equipment is comparatively less expensive than other instruments like ICP fairly new and simple and can be handled and maintained easily.
6. This technique currently employed for elemental analysis and its application includes Environmental, Biological, Metallurgical, Geological, Agricultural and Semi-conductor studies. This may be used as an effective tool for the analysis of the composition of alloys.

Although, XRF is a very useful technique yet it has its own limitations. They are described below:

1. The lowest Z-element that can be detected is around 11, because of the absorption of the low energy x-rays in the sample and in the Be window and also because of the low fluorescence yield for the low Z-elements.
2. For some elements the k-x-rays peak of certrain low Z-elements coincides with L-x-rays of the higher Z-elements. For these cases, one could look for the k-x-rays of the higher Z -elements also. Under these circumstances, the elements may be identified unambiguously by making use of sources of different excitation energies.
3. With the presently available energy resolution (170eV for 6.4KeV) it is not possible to resolve K_{α} of (Z+1) element from the K_{β} of Z^{th} element where $16 < Z < 28$.

The three techniques namely AAS, ICP-OES and XRF have been used by us for our analysis. As mentioned in this chapter, these techniques have their own advantages and disadvantages. The comparison of the three techniques based on some parameters like accuracy, sensitivity and range etc are shown below:-

COMPARISON OF THREE INSTRUMENTAL TECHNIQUES USED FOR OUR ELEMENTAL ANALYSIS

	AAS	ICP - OES	XRF
speed	Fast	slow	Fast
Minimum sample	few hundredth mg	1mg/litre	mg
Accuracy	excellent	good	excellent
Sensitivity	good	fair	fair
Range	most elements	Almost all elements	Atomic. No greater than 9
Quantitation	easy for single element	easy for simultaneous analysis	fairly easy
Price range	*Rs 6 lakhs*	*Rs 30 lakhs*	*Rs 4-5 lakhs*

Moreover, In addition to the said parameters, the selection of the technique to be used also depends on the kind of samples, the elements of interest and the availability of the facilities. Among the three techniques, we preferred AAS for our work because of its high degree of freedom from interference and highly specific. Though XRF offers a simultaneous analysis, but the degree of homogenity of the sample used is less compared to that of AAS. Also, for our soil analysis, we want to determine the element concentration in the available form while XRF gives only total content of the element. When compared with ICP-OES, the cost of analysis by AAS is cheaper. Hence AAS is more suitable for the type of work undertaken by us.

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CHAPTER-III

TRACE ELEMENTS IN ACID ALFISOLS UNDER RICE CULTIVATION

3.1 INTRODUCTION

In Meghalaya rice is grown under different agro-climatic conditions. The varying degree of laterization, podolization in the areas with sub-temperate to temperate climate and intense leaching of bases under high rainfall and steep slopes are conducive to the development of acid soils. The growth of rice depends largely on mineral nutrients which are simple inorganic compounds of a few elements. Among the elements, there are major elements like N, P, K which are used in relatively large quantities by the plant and are provided largely by the use of fertilizers. Other elements like Fe, Mn, Cu, Zn, Mo, and B are required in small amounts and are known as Micronutrients or Trace Elements [1,2]. It has been reported [3] that these trace elements either involve in metabolic processes or are constitutents of plant enzymes that regulate many vital reactions necessary for the growth, development and reproductory functions in plants. To determine the presence of the trace elements in plants, soil analysis is used as a diagnostic tool to give a priliminary idea about their deficiency or excessive concentration. Earlier studies on rice soils of our state were performed and restricted only to a few elements, namely Zn, Cu and Mn [4]. The lack of information on the status of many other trace elments in rice fields necessitated us to undertake the present study. Thus, this study was conducted with the objective to determine:-

(i) the physico-chemical properties of the soils

(ii) the trace elements contents viz: B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo and Cd of the rice soils from three different altitude zones and their interrelationship.

3.2 MATERIAL AND METHODS

(a) Sample collection :

Soil samples from 20cm depth were collected from each of the seven locations at three randomly selected sites for each altitude zone. There were total 136 samples representing the selected rice growing areas at different altitudes. Brief characteristics of the soils are given in **TABLE-1.** Air-dried and processed (< 2mm) soil samples were used for trace elements analysis.

(b) Method of analysis:

(i) **Mechanical analysis:** Mechanical analysis of soil for soil texture was determined by International Pipette method [5]. The USDA textural triangle was used for textural classification of the soil.

(ii) **Soil reaction (pH):** The pH of the soil was determined by digital pH meter having glass electrodes and a reference electrode separately with soil water ratio 1:2.5 [6].

(iii) **Organic carbon:** Organic carbon of soil was determined by Walkley and Black's titration method as described by Piper, 1950 [5].

(iv) **Cation exchange capacity:** Cation Exchange Capacity of soil was determined using neutral normal Ammonium acetate (pH 7.0) as described by Jackson [6].

(v) **Trace element analysis**

A brief discussion of chemical analysis is given below:-

(i) To measure the quantity of available trace elements in soils, DTPA-$CaCl_2$ (pH 7.3) extractant was used as described by Lindsay and Norvell [7]. The test was used for the estimation of available trace elements viz. Zn, Cu, Fe, Mn, Co, Ni, Cd, Cr, V and Se.

(ii) **Aluminium :** Aluminium was determined using NaOAc solution pH 3.8 as described by Hesse [8].

(iii) **Boron :** Available Boron was extracted with hot water as described by Wear [9].

(iv) **Molybdenum :** Molybdenum was determined by ammonium oxalate as described by Grigg [10].

Before analysing the soil samples, NBS soil samples were analysed for the same trace elements by following the same procedure for each element as mentioned above.

The results given by the NBS samples did agree with the supplied values within the statistical error. The enhancement of the results which might be contributed due to impurities present in the chemicals used were taken care by running a blank for each set of experiment performed.

3.3 RESULTS AND DISCUSSION

Physico-chemical properties:

It is evident from **TABLE-1** that soils of Meghalaya under rice cultivation are acidic in reaction with pH ranging from 4.7-5.9, and are fairly rich in organic matter with CEC varying from 13.5-73.9 Cmoles (p^+) Kg^{-1}. The organic matter ranges from 12.9 to 20.1 g/Kg and clay content varies from 11.9 to 43.5 per cent.

Trace elements:

The results of different trace elements are given in **TABLE-2 (a and b)**. The salient features of analysis for each element are highlighted below:

BORON: The average amount of Boron detected in all the rice soils ranges from 0.20 to 1.9 ppm. In the the state of Madhya Pradesh, the amount is reported to range from 0.15 to 0.66 ppm [11]. Considering 0.5 ppm as the critical limit of hot water soluble Boron in soil, this would indicate that insufficient Boron available in many of the rice growing soils of Meghalaya. Boron concentration is found to be more at lower altitudes.

ALUMINIUM: The average concentration of this element in the Meghalaya rice soil ranges from 4.0-14.0 ppm. In the state of Tamil Nadu, the KCl extractable Aluminium for soils ranges from 25-84 ppm [12]. The concentration of this element was found to be more at altitudes higher than 925 m(msl).

VANADIUM: The average concentration of this element in Meghalaya rice soil is in the range of 6.0-9.0 ppm. No change of Vanadium content is found with altitudes.

CHROMIUM: The average concentration of this element in rice soil ranges from 0.40-1.60 ppm while for the soils of the state of Uttar Pradesh, the range is reported to be from 0.25-5.01 ppm [13]. This element shows no variation with altitudes.

MANGANESE: The results obtained show that the average Manganese content of the analysed soils ranges from 9.0-143.0 ppm. This is comparable to that reported by previous workers [4] for the soils of Meghalaya which ranges from 3.0-162.0 ppm. In some acid soils of the neighbouring state like Assam, Mn ranges from 1.8-274.0 [14]. Hazra and Biswapati (1988) reported that Mn concentration in the state of West Bengal ranges from 3.1-33.3 ppm [15]. Considering 2.0 ppm as the critical limit of DTPA-extractable Mn in the soil, it shows that Mn is present in sufficient amount in the rice soils of Meghalaya. The concentration of this element is higher at lower altitudes (less than 750m,msl) except at 1550m, msl. Since Organic matter complexes manganese [16], the soils of the higher altitude with high organic matter content than those of lower altitude showed lower amounts of available Mn [17].

IRON: The average concentration of Iron in the rice soils of Meghalaya ranges from 37.0-72.0 ppm. In the state of West Bengal, the amount ranged from 16.5-114.1 ppm [15] while in Assam, the range is 6.6-1480 ppm [14]. Taking 4.5 ppm as the critical limit of DTPA-extractable Fe in the soil, the rice soils of Meghalaya have adequate amount of available Fe in the soil. Although Fe is an essential plant nutrient, involved in promoting rice root development at low concentrations, excess of water soluble Fe in soils retards rice growth. Fe toxicity occurs frequently in rice grown in poor sandy soils, in valleys or on foot hill slopes where adjacent upland soils have laterite horizons [18]. The DTPA-Fe contents of the soils do not show much variation with altitude though it is little more at altitudes 200m,msl and 550m,msl.

COBALT: The average content of Cobalt in rice soils of Meghalaya is in the range of 0.04 - 0.10 ppm. The amount detected in the state of Gujarat is reported to range from 0.12-2.10 ppm [19]. This element shows no variation with altitudes.

NICKEL: The average concentration of Nickel in rice soils of Meghalaya ranges from 0.20 - 0.50 ppm. Nickel shows no variation with altitudes.

COPPER: The average concentration of Copper in the rice soils of Meghalaya ranges from 4.0-15.0 ppm. This is higher than the amount reported by the previous workers [4] which ranges from 0.6-3.0 ppm. Some acid soils of Assam show that the range of concentration of this element is 0.30-21.52 ppm [14]. Copper in the soils of West

Bengal ranged from 2.0-10.8 ppm [15]. Taking 0.2 ppm as the critical limit of DTPA-extractable Cu in the soil [1], this element is present in adequate amoumt in rice soils of Meghalaya. The concentration of this element is higher at altitudes less than 1350m,msl.

ZINC: The average concentration of zinc detected in all the soils ranges from 0.50- 1.20 ppm which is at variance with the amount reported by Pati Ram et al [4] viz 0.7-3.3 ppm. Zn deficiency in the soils of Meghalaya would then seem to be more severe than reported earlier. In the soils of West Bengal, Zn ranges from 4.2-11.3 ppm [15]. The acid soils of Assam show a wide range of Zn concentration which is 0.08-40.2 ppm [14]. Considering 0.7 ppm as the critical limit of DTPA-extractable Zn [20] in the soil, it indicates that the concentration of this element present in 50 per cent of the rice soils of Meghalaya is insufficient. The requirement of this element is reported to be higher during the early growth stages of the plant [21]. The concentration of this element is more at altitudes less than 925m,msl.

SELENIUM: The average concentration of this element in the rice soils of Meghalaya is found to be in the range of 2.0-12.0 ppm. In the state of Uttar Pradesh, the range of available selenium is reported to be in the range of 4.0-37 ppm [22]. Hence, the soils of Meghalaya have less content of Se compared to that of Uttar Pradesh. The concentration of this element shows no variation with altitude.

MOLYBDENUM: The average concentration of Molybdenum in the rice soils of Meghalaya ranged from 0.50-2.90 ppm. This is much more than in the state of Himachal Pradesh, where it was reported to be in the range of 0.02-0.4 ppm [23]. No variation in the content of this element is found with altitude.

CADMIUM: The average concentration of Cadmium in the Meghalaya rice soils ranges from 0.02-0.20 ppm. The amount is less than the soils of Uttar Pradesh which ranges from 0.18-3.90 ppm [13]. It is also reported that in some sewage irrigated Indian soils, the content of Cd ranges from 0.10-4.0 ppm [24]. Cadmium is toxic to both plants and animals. This element also shows no variation with altitude.

The results in **TABLE-2(a, & b)** indicate some variation among soils belonging to different altitudes so far as the availability of trace elements (Zn, Fe, Al, Mn

and Cu) are concerned. Mean DTPA Manganese, Iron and Copper varied from 9-143, 37-72 and 4-15 ppm respectively. Considering 2.0, 4.5 and 0.2 ppm as the critical limits for these elements in the soils [25], these nutrients are sufficient in most of the analysed soils of the lower altitude. Also Copper and Manganese are found to be high at altitude less than 1350m,msl and 750m,msl, respectively.

The element Zinc varied from 0.5-1.2 ppm. Taking 0.7ppm as the critical limit in rice soils [20], 50 percent of the rice soils are likely to be deficient in Zinc at altitudes above 925m, msl.

The concentrations of DTPA Cobalt, Chromium, Selenium and Cadmium, when compared with reported values, are comparatively low in these soils, ranging from 0.04-0.1, 0.4-1.6, 2.0-12.0 and 0.02-0.20 ppm, respectively. Nickel, Vanadium and percent Aluminium saturation varies from 0.20-0.50, 6.0-9.0 and 4.0-14.0 ppm respectively. Vanadium and Aluminium may be considered to be adequate while Nickel may be considered to be less in the rice soils. Mean Hot water Boron and ammonium oxalate extracted Molybdenum range from 0.2-1.9 and 0.5-2.9 ppm, respectively. Considering 0.5 ppm soil as a critical limit of Hot water soluble Boron in soil, 35 percent of the analysed rice soils are likely to be deficient. But ammonium oxalate extracted Molybdenum is sufficient in these soils.

The worked out correlation between the selected physico-chemical properties and the three different altitudnal zones is shown in **TABLE-3**. It is observed that below 700m, msl, the CEC and the clay show a significant negative correlation of values r = -0.47 and r = -0.82 respectively while at mid-altitude (701-1200m,msl), the CEC shows a positive correlation (r = 0.59). At the altitudinal zone greater than 1200m,msl, only Organic carbon shows a significant negative correlation of r = -0.49.

The correlation of the concentrations of different elements with the three altitudinal zones (<700m,msl; 701-1200m,msl and >1200m,msl) is shown in **TABLE-4**. The elements Copper (0.49), Molybdenum (0.50), Boron (0.41), Zinc (0.45) and Manganese (0.44) show a positive correlation only with low altitude (less than 700m,msl) but negative with higher altitudes. The other elements like Vanadium,

Chromium, Iron, Cobalt, Nickel, Selenium and Cadmium show no significant correlation with altitude.

The correlations between element to element are given in TABLE-5. The correlation study showed the significant negative correlation of the element Aluminium with some of the elements like Manganese (r = -0.52), Copper (r = -0.43) and Zinc (r = -0.44). Manganese manifests a positive correlation with Cobalt (r = 0.42), Copper (r = 0.41) and Zinc (r = 0.43). Iron like Manganese has a significant positive correlation with Zinc (r = 0.53) and Copper (r = 0.41). Copper and Boron also exhibit a positive correlation with Zinc (r = 0.82) and Aluminium (r = 0.42) respectively. The correlations between other elements are not statistically significant.

Our correlation studies support the finding of Patrick and Reddy [1] that the chemistry of Copper in flooded soils is similar to Zinc. At low concentration Zinc and Copper has synergestic effect and at high concentration, antagonism between them may occur. Similar effect may take place between Zinc and Iron [26]. Acidic soils generally show toxic effect of Iron for paddy under reduced conditions, which in turn reduced the availability of Manganese. Also, the negative correlation between Manganese and Aluminium suggests the antagonistic effect between them.

CONCLUSION:

Thus, these soils have low content of **Boron, Zinc, Cobalt, Nickel, Chromium, Selenium** and **Cadmium** but adequate amount of **Vanadium, Iron, Aluminium, Copper, Manganese** and **Molybdenum.** Results suggest the need for a closer monitoring of nutrients, especially **Boron and Zinc in acid alfisols of Meghalaya** under rice cultivation, besides the major plant nutrients to enhance rice production in the state.

TABLE-1

SOME PHYSICO-CHEMICAL PROPERTIES OF THE SOILS

SOIL LOCATION	ALTITUDE m,msl	pH	ORGANIC CARBON g Kg^{-1}	CEC cmol(p+) Kg^{-1}	SAND (%)	SILT (%)	CLAY (%)
BYRNIHAT	**200**	5.5-5.9 (5.7)	17.9-20.1 (19.0)	35.3-50.7 (43)	28.7-35.3 (32)	28.5-33.4 (31)	34.4-39.5 (37)
NONGPOH	**550**	5.1-5.8 (5.5)	18.6-19.4 (19.0)	28.9-39.1 (34)	46.8-55.2 (51)	17.9-28.1 (23)	23.8-28.2 (26)
UMSNING	**750**	5.1-5.7 (5.4)	16.5-17.5 (17.0)	13.5-26.5 (20)	24.5-31.5 (28)	42.0-46.0 (44)	25.2-30.8 (28)
SUMER	**925**	4.9-5.5 (5.2)	17.3-18.8 (18.0)	30.2-59.8 (45)	23.2-58.8 (41)	21.5-46.5 (34)	11.9-38.0 (25)
RYMPHUM RES.FARM	**1350**	5.0-5.4 (5.2)	18.3-19.6 (19.0)	45.0-54.9 (50)	24.1-39.9 (32)	30.0-46.0 (38)	16.5-43.5 (30)
LANGTOR	**1550**	4.9-5.4 (5.1)	14.5-15.5 (15.0)	36.0-73.9 (55)	28.0-50.0 (39)	22.7-44.3 (33)	15.2-40.8 (28)
MYLLIEM	**1600**	4.7-5.3 (5.0)	12.9-15.1 (14.0)	42.6-67.4 (55)	33.1-48.9 (41)	24.0-51.9 (38)	13.6-28.4 (21)

TABLE - 2a

AVAILABLE MICRONUTRIENT ELEMENTS IN THE SOILS

Soil location	Altitudes m,msl	No. of soil samples	Mn	Fe	Cu	Zn	V	Co	Ni
Byrnihat	**200**	23	70 - 169	30 - 99	9 - 21	0 7-1 7	8 - 11	0 04-0 2	0 2-1 0
			(143)	(61)	(15)	(1 2)	(9)	(0 1)	(0 4)
Nongpoh	**550**	25	12 - 87	45 - 92	5 - 37	0 5-2 8	0 4 - 11	0 02-0 1	0 3-0 6
			(44)	(72)	(12)	(1 0)	(9)	(0 06)	(0 3)
Umsning	**750**	15	21 - 117	36 - 64	4 - 16	0 6-1 4	8 - 11	0 02-0 07	0 2-0 6
			(56)	(49)	(9)	(0 9)	(9)	(0 04)	(0 4)
Sumer	**925**	15	5 - 24	41 - 49	12 - 17	0 3-0 6	6 - 11	0 04-0 1	0 3-0 6
			(15)	(45)	(14)	(0 5)	(8)	(0 07)	(0 5)
Rymphum Res. Farm	**1350**	12	4 - 16	29 - 67	1 - 5	0 5-1 0	0 02- 8	0 01-0 07	0 2-0 3
			(9)	(50)	(4)	(0 7)	(6)	(0 04)	(0 2)
Langtor	**1550**	11	56 - 76	34 - 40	4 - 7	0 5-0 8	0 02 - 7	0 03-0 07	0 3-0 6
			(66)	(37)	(5)	(0 6)	(6)	(0 06)	(0 4)
Mylliem	**1600**	35	7 - 90	26 - 73	3 - 12	0 5-1 1	0 4 - 11	0 02-0 07	0 2-0 6
			(24)	(51)	(6)	(0 7)	(9)	(0 04)	(0 4)

Note:- Figure in parenthesis denotes mean

TABLE - 2b

AVAILABLE MICRONUTRIENT ELEMENTS IN THE SOILS

Soil location sites	Altitudes (m)	No. of soil samples	B	Al	Mo	Cr	Se	Cd
Byrnihat	200	23	0 1-2 4	3 - 5	0 8-2 0	0 5-1 5	2 - 8	0 05-0 2
			(1 1)	(4)	(1 5)	(1 0)	(3)	(0 1)
Nongpoh	550	25	1 7-2 2	3 - 8	0 8-2 3	0 5-2 0	0 8 - 3	0 01-0 2
			(1 9)	(5)	(1 3)	(0 9)	(2)	(0 1)
Umsning	750	15	0 1-0 3	3 - 4	0 4-1 1	0 5-2 5	1 - 5	0 00-0 05
			(0 2)	(4)	(0 6)	(1 4)	(3)	(0 02)
Sumer	925	15	0 01-0 6	10 - 13	1 6-4 0	0 5-1 5	2 - 8	0 05-0 1
			(0 4)	(12)	(2 9)	(0 7)	(4)	(0 08)
Rymphum Res. Farm	1350	12	0 5-0 7	12 - 18	0 3-0 8	0 2-0 9	8 - 17	0 1-0 3
			(0 6)	(14)	(0 6)	(0 4)	(12)	(0 2)
Langtor	1550	11	0 8-1 05	12 - 16 5	0 05-1 05	0 5-1 2	2 - 3	0 00-0 1
			(0 9)	(13)	(0 5)	(0 7)	(2)	(0 05)
Mylliem	1600	35	0 3-0 5	11 - 14	0 4-1 5	0 5-3 0	2 - 11	0 05-0 20
			(O 4)	(12)	(1 1)	(1 6)	(4)	(0 1)

Note:- Figure in parenthesis denotes mean

TABLE-3

CORRELATION BETWEEN PHYSICO-CHEMICAL PROPERTIES OF THE RICE SOILS AND VARYING ALTITUDES

Physico-chemical Properties	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
pH	0.30	0.08	- 0.09
Organic carbon	- 0.04	0.30	- 0.49*
CEC	- 0.47*	0.59**	0.18
Clay	- 0.82**	- 0.17	- 0.25
	r^2 = 0.917	r^2 = 0.462	r^2 = 0.554

*Significant at $p < 0.05$, **Significant at $p < 0.01$

TABLE-4

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS OF RICE SOILS AND ALTITUDE

Trace elements Conc.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	0.41*	- 0.37	- 0.28
Aluminium	- 0.21	- 0.25	0.41*
Vanadium	0.11	- 0.13	- 0.09
Chromium	- 0.27	0.22	0.24
Manganese	0.44*	- 0.27	- 0.29
Iron	0.36	- 0.26	- 0.31
Cobalt	0.29	- 0.21	- 0.18
Nickel	0.04	- 0.05	- 0.08
Copper	0.49*	- 0.39	- 0.32
Zinc	0.45*	- 0.18	- 0.14
Selenium	- 0.29	0.16	0.12
Molybdenum	0.50*	- 0.35	- 0.30
Cadmium	0.03	- 0.06	- 0.08
	$r^2 = 0.856$	$r^2 = 0.874$	$r^2 = 0.883$

*Significant at $p < 0.05$

TABLE-5

CORRELATION COFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE SOILS OF MEGHALAYA

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.42*	0.12	-0.26	0.28	0.33	0.28	-0.02	0.21	0.28	-0.16	0.11	0.11
Al		1	-0.35	-0.02	-0.52*	-0.32	-0.33	-0.09	-0.43*	-0.44*	-0.25	-0.19	0.02
V			1	0.16	0.14	0.26	-0.03	0.08	0.32	0.27	-0.21	0.15	0.004
Cr				1	-0.09	-0.05	-0.11	0.08	-0.12	-0.01	-0.14	-0.14	-0.07
Mn					1	0.16	0.42*	0.21	0.41*	0.43*	-0.20	0.18	-0.15
Fe						1	0.21	-0.04	0.41*	0.53*	-0.25	0.09	0.05
Co							1	0.18	0.25	0.13	-0.09	0.31	-0.13
Ni								1	0.03	0.06	-0.15	-0.11	-0.36
Cu									1	0.82**	-0.24	-0.29	-0.12
Zn										1	-0.19	-0.03	0.03
Se											1	-0.11	0.04
Mo												1	1
Cd													1

*Significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.794$

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CHAPTER-IV

DISTRIBUTION OF TRACE ELEMENTS IN RICE PLANTS AT DIFFERENT GROWTH STAGES

4.1 INTRODUCTION

Analysis of the soils under rice cultivation showed that the element like V, Al, Mn, Cu, Mo and Fe are present in adequate amounts while concentrations of other elements like Zn, B, Cr, Co, Ni, Se and Cd were low. Among the known trace elements essential for the growth of the plants are Fe, Zn, Cu, Mn, and Mo. Others like Co, Se, and V appear to be helpful for growth of certain species [1-3]. The functions of the trace elements are mainly catalytic and as constituents of metallo-enzymes. The amount of nutrients absorbed from the soil by the plant depends upon the variety of the plant and its stages of growth. **APPENDIX-V** shows the concentration of a few trace elements (micronutrients) in plants [4].

Plants usually exhibit external symptoms as a result of trace element imbalances but sometimes these symptoms are similar for different elements or they are suppressed altogether except that the yield is low. Soil tests are initially used to predict the deficiency/toxicity of the trace elements in the plant. However, a thorough idea about the status of trace elements is obtained by carrying out the total plant analysis, a diagnostic tool for determining deficient, sufficient or excessive amounts of essential elements in plant. In this chapter, we present the total rice plant analysis at different growth stages. This gives the information about the concentrations of the trace elements present in the plant and hence heir deficiency/toxicity can be ascertained.

4.2 PLANT ANALYSIS

A total of 210 plant samples were collected at different stages of growth such as seedling, tillering, panicle and at maturity from the same fields as were the soil samples. The whole plant was sampled at all growth stages. The variety chosen for our study was "NONGLWAI" (local name), a local variety grown extensively in the state. A survey

indicated that the farmers of this state prefer to grow local varieties as the demand for them is much higher than other improved varieties. Details of the collection sites are given in **TABLE-6.**

Analysis of Trace Elements:

The plant tissue was washed properly in dilute water, 0.01N HCl and finally by triple distilled water. In mature plants, grain and straw were separated and also washed. Plant tissues were dried in a hot air circulation oven at 50° C . The dried samples were ground to fine powder and weighed. About 0.5gm of the fine powder sample was digested with a triacid mixture (HNO_3:H_2 SO_4 :$HCLO_4$ :: 3:1:1) at 65° C. (all acids used were of analytical grade). It was then filtered through Whatman filter and the final volume was made up to 50ml and analysed for trace elements.

For the determination of B, a separate digestion of the samples was carried out in Teflon cups to avoid the additive effect of B present in the glasswares. Following the same procedure, NBS samples(Citrus leaves and Pine needles) were analysed under the same conditions. The concentration of the elements were detected by using an **Atomic Absorption spectrometer (AAS), (Perkin Elmer Model 2380) and an Inductively Coupled Plasma-optical emission spectrophotometer (ICP-OES), (LABTAM 8440M Model). XRF** was also used as a complementary technique. The samples were analysed for Al, Zn, Fe, V, Cr, Mn, Co, B, Mo, Ni, Cu, Se and Cd. The enhancement of the results which might be resulted due to impurities present in the chemicals used had been taken care by running a blank for each sets of experiment performed. The results given by the NBS samples did agree with the supplied values within the statistical error. This shows that the method used for digesting our samples was suitable for the analysis.

4.3 RESULTS AND DISCUSSION

The results of the distribution of the trace elements in rice plant at different growth stages with respect to altitude are given in **TABLE-7 (a, b & c)**. The detail of each element is discussed below:

BORON: Among anionic micronutrients B content of rice plant ranges from 33.0 to 36.0 ppm at seedling, 23.0 to 27.1 ppm at tillering, 20.0 to 21.9 ppm at panicle initiation, 5.0 to 6.0 ppm in straw and 3.0 to 5.0 ppm in grain. Takkar and Randhawa [5] reported that 18.0 ppm is the critical limit of boron in rice plant at tillering stage It was reported [6] that boron concentration in grain ranged from 1.2-4.0 ppm while in straw it ranged from 2.0-6.0 ppm. Tanaka and Yoshida [7] reported that 3.4 ppm in straw at growth stages showed deficiency and at 100.0 ppm in straw at maturity indicated toxicity to the plant.

ALUMINIUM: The Al content in rice plant varies from 324.7 to 337.0 ppm at seedling stage, 304.0 to 319.6 ppm at tillering stage, 226.0 to 261.7 ppm at panicle initiation, 66.0 to 71.0 ppm in straw and 10.6 to 27.4 ppm in grain. Tanaka and Yoshida [7] reported that 300.0 ppm is the critical concentration of Aluminium at tillering stage. According to Sarkunan and Biddappa [8], the concentration of Aluminium in grain and straw is 30.0 ppm and 85.0 ppm. Tanaka and Navasero [9] found that 25.0 ppm is the critical concentration of Aluminium for rice. Howeler and Cadavid [10] reported that 3.0 ppm Aluminium increased the root growth and grain yield.

VANADIUM: The content of V in rice plant ranges from 0.47 to 0.56 ppm at seedling stage, 0.36 to 0.42 ppm at tillering stage, 0.21 to 0.24 ppm at panicle initiation stage, 0.05 to 0.07 ppm in straw and 0.02 in grain. Other workers [7] reported it to be less than 0.02 ppm in grain and less than 0.04 ppm in straw.

CHROMIUM: The Cr content of rice varies from 0.74 to 1.74 ppm at seedling, 0.68 to 1.40 ppm at tillering, 0.43 to 0.57 ppm at the panicle initiation stage, 0.08 to 0.16 ppm in straw and 0.02 to 0.05 ppm in grain. Others workers [11] reported it to be less than 0.005 ppm in grain and less than 0.12 ppm in straw.

MANGANESE: The total Mn content of rice plant varies from 2635.0 to 2713.0 ppm at seedling, 2556.0 to 2665.0 ppm at tillering, 2445.0 to 2545.0 ppm at panicle initiation, 375.0 to 465.0 ppm in straw and 94.0 to 115.0 ppm in grain. Tanaka and Yoshida [7] reported that more than 2500.0 ppm in the shoot of rice plant at tillering stage may produce toxicity to the plant. Sarkar and Deb [12] reported to be 32.0-90.0 ppm in grain and 155.0-375.0 ppm in straw. Other workers [11] reported that the concentration of Mn

in grain was similar ranging from 26.0-80.0 ppm while in straw its range was lower from 70.0-150.0 ppm.

IRON: The total Fe content of rice plant varies from 332.9 to 381.0 ppm at seedling, 313.2 to 347.0 ppm at tillering, 282.0 to 318.0 ppm at panicle initiation, 172.0 to 285.0 ppm in straw and 50.0 to 120.0 ppm in grain. Tanaka and Yoshida [7] reported that 70.0 ppm is the critical limit for the deficiency while more than 300 ppm indicates toxicity to the rice plant at tillering stage. Others [12] reported that Fe concentration in grain ranged from 20.0-1720.0 ppm while in straw it ranged from 400.0-1960.0 ppm.

COBALT: The Co content of rice ranges from 0.43 to 0.69 ppm at seedling, 0.34 to 0.52 ppm at tillering stage, 0.23 to 0.35 ppm at panicle initiation stage, 0.10 to 0.16 ppm in straw and about 0.05 ppm in grain. It was reported [11] that Co concentration in grain is less than 0.03 ppm while in straw it was less than 0.08 ppm.

NICKEL: The Ni content ranges from 0.94 to 1.19 ppm at seedling stage, 0.70 to 0.74 ppm at tillering stage, 0.38 to 0.49 ppm at panicle initiation stage, 0.41 to 0.48 ppm in straw and 0.25 to 0.28 ppm in grain. Other workers [11] found that Ni concentration in grain and straw ranged from 0.27-4.00 and 0.33-0.97 ppm.

COPPER: The Cu content in rice plant varies from 21.0 to 24.0 ppm at seedling, 16.0 to 19.0 ppm at tillering, 8.0 to 15.0 ppm at panicle initiation, 3.0 to 9.7 ppm in straw and 4.0 to 6.0 ppm in grain. It was reported [13] that rice leaves at tillering stage show deficeincy of Cu at 2.0-5.0 ppm and 5.0 ppm indicates sufficiency of the element. However, 40.0 ppm showed toxicity to the plant. Others reported [14] Cu concentration in most plants, including, rice ranged from 2.0-20.0 ppm and the ciritical limit was set at below 10.0 ppm. Sarkar and Deb [12] reported it to be below 4.0-10.0 ppm in grain and 4.0-11.0 ppm in straw similar to the finding of other workers [6].

ZINC: Among cationic micronutrients, total Zn content of rice plant ranges from 12.0 to 13.0 ppm at seedling stage, 8.0 to 10.0 ppm at the tillering stage, 5.0 to 7.2 ppm at panicle initiation stage, 20.0 to 22.0 ppm in straw and 9.9 to 18.0 ppm in grain. The levels of Zinc at or below which rice showed deficiency symptoms or gave a significance response had been established by several workers. These data reveal that the limit for growing rice was around 15.0 ppm [14]. Mishra et al [6] reported to be 21.0-

41.0 ppm in grain and 63.0-123.0 ppm in straw which was much higher compared to our findings. Comparing to the mentioned reported data, it indicates that the rice plant suffers deficiency of this element at all growth stages.

SELENIUM: The Se content varies from 1.39 to 1.74 ppm, 0.93 to 1.40 ppm, around 0.57 to 0.63 ppm, 0.07 to 0.16 ppm and 0.02 to 0.05 ppm at seedling, tillering, panicle initiation, straw and grain stages respectively.

MOLYBDENUM: The Mo content varies from 0.58 to 0.74 ppm at seedling stage, about 0.54 ppm at tillering, 0.35 to 0.42 ppm at panicle initiation stage, 0.22 to 0.30 ppm in straw and 0.14 to 0.20 ppm in grain. Takkar and Randhawa [5] reported that the critical limit of Molybdenum at the tillering stage is 1.8 ppm. Misra et al [6] reported to be 0.05-0.22 ppm in grain and 0.11-0.55 ppm in straw.

CADMIUM: The Cd content varies from 0.35 to 0.42 ppm, 0.27 to 0.33 ppm, 0.18 to 0.23 ppm, around 0.04 ppm and about 0.02 ppm at the seedling, tillering, panicle initiation straw and grain stages respectively. Cadmium is closely associated with Zinc both in occurance and metabolism. Honma and Hirata [15] observed the increase of Cadmium absorption and its transport in Zinc deficient rice plant.

At the mid-altitude, the contents of the elements **Vanadium, Cobalt**, and **Copper** tended to be more while **Chromium, Boron** and **Aluminium** show low content. At the tillering stage, the elements **Iron, Selenium** and **Cadmium** tended to be more at higher altitude. The concentrations of elements **Manganese** and **Nickel** decrease with increase of altitude at the initial growth stages. For elements **Molybdenum** and **Zinc**, a negative correlation is observed with mid-altitude though no variation is shown in grain and straw by **Molybdenum**. The element **Zinc** shows a positive correlation in straw at mid-altitude and in grain at low altitude.

The concentrations of elements **Boron, Copper** and **Molybdenum** at tillering stage are compared with their critical values. It is found that Boron concentration (25.6 ppm) is higher than the critical limit by 18.0 ppm at the tillering stage [5]. Copper content is found to be around 17.0 ppm. This value lies between 5.0 ppm, which shows sufficiency and 40.0 ppm which indicates toxicity [13]. For Molybdenum, the

concentration (0.54 ppm) is found to be less when compared with the critical limit which is 1.8 ppm [5].

The concentrations of the elements like **Vanadium** and **Nickel** in grain and straw are similar to the values reported by the previous workers [7, 11]. The contents of **Chromium** and **Cobalt** in grain and straw are slightly higher than the reported values [11]. It may therefore be considered that the concentrations of these four elements (Vanadium, Nickel, Chromium, Cobalt) are adequate for the normal growth of the rice plant.

The concentrations of elements **Aluminium, Manganese** and **Iron**, at the tillering stage are found to be higher than the reported critical limits [7] which are 300 ppm for both Aluminium and Iron and 2500 ppm for Manganese.

As the soils of Meghalaya are acidic in nature, the solubility of **Aluminium, Manganese** and **Iron** is high and these elements are present in adequate amount and in available form which the plant can utilise. Kinraide [16] reported that Aluminium is present as complex Aluminosilicates in acid soils thereby releasing Aluminium in the form of hexaaqua-aluminium $[Al(H_2O)_6]$ called Al^{3+} which is detrimental to plants and limits the growth and productivity. Moreover, Aluminium is known to reduce the uptake and transport of many essential elements which has been given higher priority. Although both Manganese and Iron at low concentrations are essential for plant nutrients involved in promoting rice roots development but excess of these elements may retard rice growth [17]. A wide variation found for Iron and Manganese concentrations may be due to the increased solubility of these nutrient elements under waterlogged conditions and differential absorptions by rice cultivars. The symptoms described for Iron toxicity are more or less similar to those of Zinc deficiency [12, 18].

The concentration of **Zinc** is found to be less than the reported values in all the stages including grain and straw [14, 19]. The data of the previous workers revealed that the critical limit for growing rice is around 15 ppm [14]. Zinc deficiency in rice plant is often attributed to the decreased availability of the element in soil [19]. Such decrease had been explained as being due to the precipitation of the element as oxide, hydroxide, carbonate, phosphate, sulfide, silicate etc [20]. Biswapati et.al [21] had shown that

organic complexed forms play the most important role in Zinc nutrition of lowland rice Zinc deficiency brings about physiological changes which significantly increases the Cadmium accumulation in the roots and its transport into the shoot [15].

The concentrations of **Aluminium, Manganese** and **Iron** are higher in the rice plant and may cause toxicity especially at the tillering stage when the concentrations of all three elements are found to be high. Deficiency of **Zinc** occurs in the rice plant when the concentration of this element is found to be less in all the stages. The concentrations of **Boron, Vanadium, Nickel, Chromium, Copper, Molybdenum and Cobalt** are considered to be adequate for the normal growth of the rice plant. The increase and decrease of one element with respect to the other brings about imbalances among the trace elements and their uptake and this may be one of the factors for the low yield of the crop in this state.

As expected, the content of the trace elements decreases with age, either as a result of dilution effect owing to an increase in dry matter production or a decrease in rate of its absorption from the growth medium beyond a particular growth stage. As perusal of the data in **TABLE-7(a, b & c)** vividly reveals these facts, i.e. the concentrations of all trace elements markedly decrease in different parts of the plant with increase in plant age. It is worth mentioning that many researchers studying mineral imbalances in rice have found that, when there is a deficiency of one nutrient, uptake of other nutrients with same charge is increased, while uptake of nutrients with different charges decreases [22].

CORRELATION WITH ALTITUDES

The correlation of the concentration of different elements with the three altitudinal zones (<700m,msl; 701-1200m,msl and >1200m,msl) at different growth stages are shown in **Table-8a to 8e.**

Seedling stage:

The total contents of trace elements viz: Manganese and Nickel have a significant negative correlation while Vanadium shows a significant positive correlation with altitudes above 700m,msl at the seedling stage. The concentrations of Boron,

Chromium and Zinc also show negative correlation with mid-altitude (701-1200m,msl). The total content of Molybdenum and Cobalt have a significant positive correlation with mid-altitude but negative correlation with higher altitude (>1200m,msl). Copper and Selenium concentration at this stage show significant positive correlation with all altitudes but non-significant relationship was observed with lower altitudes for Selenium. A statistically non-significant correlation is observed for all the trace elements with lower altitudes except Cobalt (r= -0.54).

Tillering stage:

The total contents of trace elements namely Boron, Aluminium, Molybdenum, Manganese and Nickel show a significant negative correlation with the mid-altitude zone (701-1200m,msl). Manganese does show similar pattern with low altitude while Nickel shows with higher altitude. The elements Vanadium and Cobalt show positive correlation with the mid-altitude but the latter gives a significant negative correlation with higher altitude. The elements Chromium shows negative correlation with mid-altitude but positive with low altitude. The element Iron and Cadmium exhibit a significant positive correlation with higher altitude only.

Panicle stage:

The total concentration of Manganese shows a significant negative correlation with all the altitudes. At higher altitudes, Molybdenum also show a negative correlation but Iron and Copper give a positive correlation. Similarly, Boron, Chromium and Molybdenum give a negative correlation with mid-altitude. At low altitude, Boron shows a negative correlation. The elements Vanadium and Cobalt exhibit a positive correlation with mid-altitude and significant negative correlation with higher altitudes.

Grain:

The total concentration of Manganese and Copper show a negative correlation with low altitudes (<700m,msl) but positive with mid-altitude. Like the previous elements, Vanadium and Iron show the similar pattern with low altitudes and also with higher altitudes for Iron. Zinc shows a positive correlation with low altitudes but negative at mid-altitudes. With higher altitudes, Cobalt shows a significant negative correlation while Selenium and Cadmium give a positive correlation.

Straw:

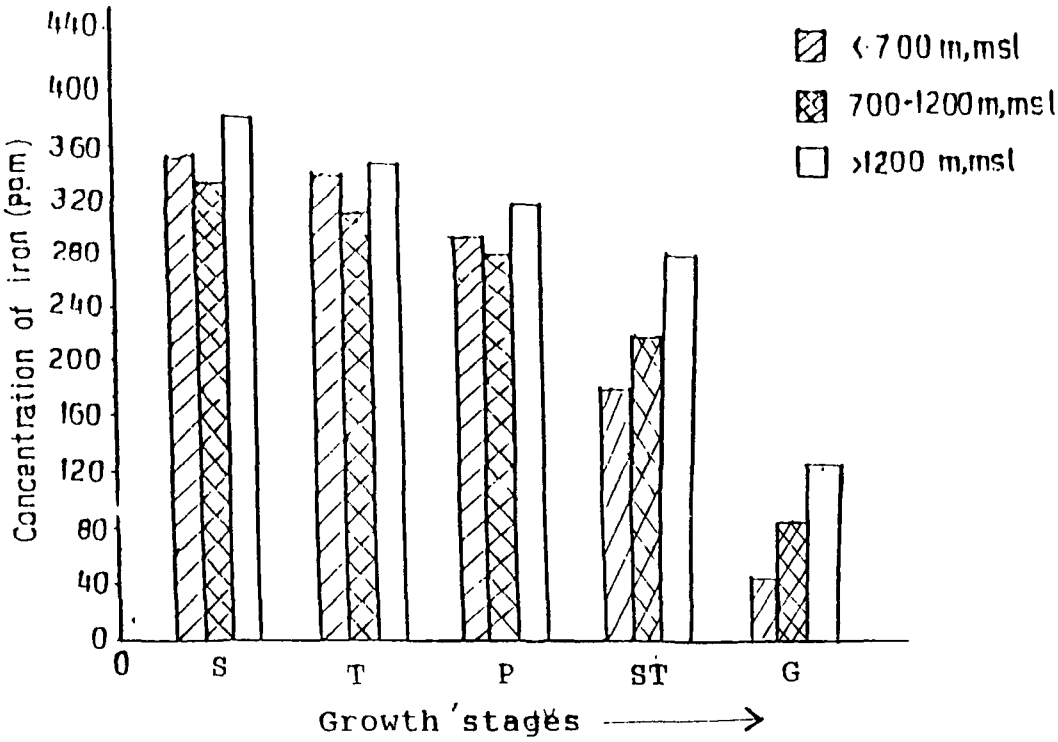
The total concentration of Manganese shows a negative correlation with low altitudes and higher altitudes but positive correlation with mid-altitude like Iron, Cobalt, and Zinc. The elements Copper and Vanadium show a significant positive correlation with mid-altitude and higher altitude. Aluminium shows a negative correlation with low altitude. Cadmium shows a negative correlation with mid-altitude and positive with higher altitude. A typical diagram on the influence of altitude on the concentration of Iron and Copper is shown in **FIG-13.**

The correlation between element to element at different growth stages are given in **Table-9a to 9e**.

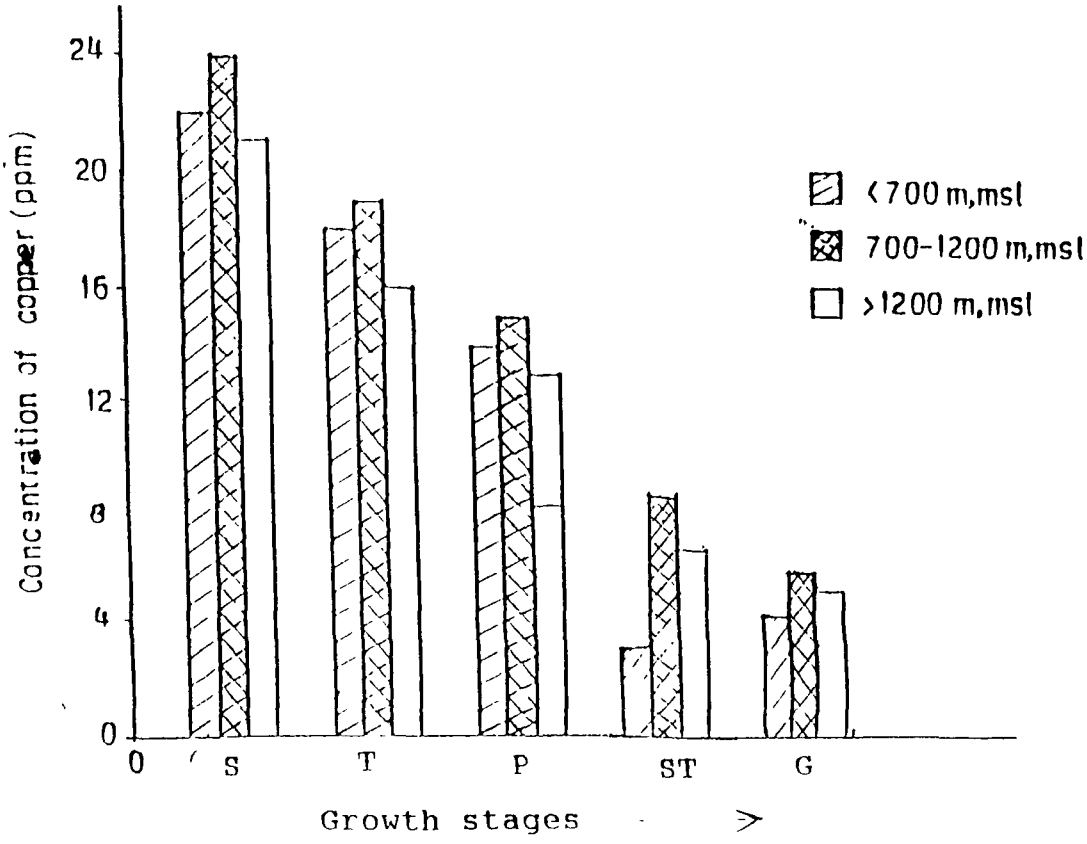
Aluminium in the rice plant shows positive correlation with Nickel at seedling stage and in grain. With Copper, it shows positive correlation at the tillering stage and in straw while Vanadium shows negative correlation at the seedling stage but positive correlation in straw with Aluminium. A positive correlation with Aluminium is observed for different elements such as Cobalt at seedling stage, Iron at panicle stage, and Molybdenum at grain stage. Manganese shows a positive correlation with Aluminium in straw only.

Vanadium is positively correlated with Iron at all stages of the rice plant except at seedling and at straw stages. With Copper, Vanadium shows a positive correlation at tillering and straw stages. Vanadium correlates with Boron in grain while with Manganese in straw. Cobalt is negatively correlated with Vanadium at seedling stage but positively correlated at panicle stage. Vanadium also correlates positively with Chromium at tillering stage and with Nickel, Selenium and Cadmium at panicle stage. Chromium shows positive correlation with Nickel at first two stages namely seedling and tillering stage. Molybdenum also shows similar pattern with Chromium in grain and straw only. Chromium is positively correlated with Manganese at tillering stage. In grain, Selenium and Cadmium give positive correlation with Chromium while Iron a negative correlation.

INFLUENCE OF ALTITUDE ON IRON CONCENTRATION OF RICE PLANT AT DIFFERENT GROWTH STAGES.



INFLUENCE OF ALTITUDE ON COPPER CONCENTRATION OF RICE PLANT AT DIFFERENT GROWTH STAGES.



(S=seedling, T=tillering, P=panicle, ST=straw, G=grain)

FIG-13. SHOWS THE INFLUENCE OF ALTITUDE ON THE CONCENTRATION OF IRON AND COPPER

Manganese is positively correlated with Cadmium at seedling, Iron and Nickel at tillering; Iron, Selenium and Cadmium at panicle; Copper and Selenium in grain and Copper in straw. In straw, Zinc shows negative correlation with Manganese.

Iron shows a positive correlation with Copper at the two initial stages i.e. seedling and tillering. At panicle stage, it gives positive correlation with elements Cobalt, Cadmium and Selenium. Moreover, it shows a positive correlation with Cobalt in grain while negative with Selenium. Nickel shows a positive correlation with Cobalt at tillering and panicle stages while with Molybdenum and Cadmium at the latter stage only.

Selenium is positively correlated with Cadmium at seedling, panicle and in grain. Copper also positively correlates with Selenium in straw. The elements Molybdenum is also positively correlated with Cadmium at seedling stage and in straw, and with Boron at tillering stage only.

The positive correlation of the element Iron with many trace elements like Vanadium, Manganese, Cobalt, Selenium and Cadmium mostly at the initial growth stages indicates that Iron concentration initially enhances the uptake of these elements more in the initial stages than in the later ones. Also, positive correlation is obtained with Cobalt in grain. The severity of Cobalt toxicity was minimised with increasing Iron supply, reflecting antagonism [23]. Enhanced uptake of Iron by the plant decreases the uptake of other essential elements necessary for other metabolic functions. With element Manganese, Iron shows a positive correlation both at tillering and panicle stages. Experiment conducted [17] showed that Manganese in the tissue increases with increasing levels of Iron. If Manganese is deficient in the plant, then there will be an excess of active Fe^{2+} iron leading to toxicity. Presence of Manganese oxidises Fe^{2+} to inactive Fe^{3+} thereby alleviating the injurous effect of active Iron in the tissue. The positive correlation of Iron with Manganese is expected as the rice soil under cultivation is acidic in nature.

Positive correlation of Nickel with Cobalt is obtained at tillering and panicle stages suggesting that synergistic effect takes place. This may be explained as Nickel and Cobalt are associated geochemically. Their atomic weights are very close (58.94 and 58.69) and hence it is expected to have similar physiological role [24]. Also, Nickel is

regarded as a very toxic element which interferes with Iron nutrition or produces other specific signs of symptoms as both of them are highly correlated with other elements [25]. At the seedling stage, Vanadium shows a negative correlation with Aluminium. This might have resulted due to the pH of the soil which favours the availability of the element Aluminium.

CONCLUSION:

The contents of **Vanadium, Copper** and **Cobalt** are tended to be more at mid-altitudes (701-1200m,msl). The elements **Iron, Selenium** and **Cadmium** are found to be more at altitudes greater than 1200m,msl while **Manganese** is more at altitude less than 700m(msl). Other trace elements like **Boron, Aluminium, Chromium, Zinc,** and **Molybdenum** are considered as no variation with altitudes.

The availability of the elements **Boron, Vanadium, Nickel, Chromium, Cobalt, Copper** and **Molybdenum** are considered to be adequate for the normal growth of the rice plant. But the concentrations of **Aluminium, Manganese** and **Iron** are found to be above the critical limits in the rice plant which may cause toxicity especially at the tillering stage. The element **Zinc** is deficient in the rice plant at all the stages This nutrient imbalance is the problem which is to be considered seriously for improving the yield. This problem which has become the threat to the farmers must be thoroughly understood with proper soil management.

TABLE-6

SITES OF PLANT SAMPLES COLLECTED WITH RESPECT TO ALTITUDES

Sl. No.	DISTRICT	SITES	No. OF SAMPLES	ALTITUDES m,msl	CROP ROTATION
1.	RI-BHOI	BYRNIHAT	40	200	rice-rice
2.	RI-BHOI	NONGPOH	40	550	rice-tomato/vegetable
3.	RI-BHOI	UMSNING	15	750	rice-tomato/vegetable
4.	RI-BHOI	SUMER	15	925	rice-tomato/vegetable
5.	JAINTIA HILLS	RYMPHUM RES.FARM	40	1350	rice-potato/vegetable
6.	WEST KHASI HILLS	LANGTOR	30	1550	rice-potato
7.	EAST KHASI HILLS	MYLLIEM	30	1600	rice-potato

TABLE-7(a)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE < 700m, msl

GROWTH STAGES	B	Al	V	Cr	Mn
Seedling	32 8-33 2 (33 0)	324 1-327 9 (326 0)	0 28-0 78 (0 53)	0 54-0 94 (0 74)	2712 0-2714 0 (2713 0)
Tillering	25 4-25 8 (25 6)	302 7-305 3 (304 0)	0 24-0 60 (0 42)	0 48-0 88 (0 68)	2659 0-2671 0 (2665 0)
Panicle initiation	20 9-21 1 (21 0)	236 4-237 6 (237 0)	0 09-0 39 (0 24)	0 33-0 53 (0 43)	2541 0-2549 0 (2545 0)
Straw	4 95-5 05 (5 0)	69 3-72 7 (71 0)	0 05-0 07 (0 06)	0 07-0 09 (0 08)	461 0-469 0 (465 0)
Grain	2 94-3 06 (3 0)	26 3-27 7 (27 0)	0 012-0 28 (0 02)	0 02-0 04 (0 03)	113 0-117 0 (115 0)

ALTITUDE 701-1200m, msl

GROWTH STAGES	B	Al	V	Cr	Mn
Seedling	35 96-36 04 (36 0)	323 4-326 0 (324 7)	0 33-0 61 (0 47)	1 64-1 84 (1 74)	2677 0-2693 0 (2685 0)
Tillering	27 06-27 14 (27 1)	318 6-320 6 (319 6)	0 16-0 56 (0 36)	1 20-1 60 (1 40)	2594 0-2606 0 (2600 0)
Panicle initiation	21 88-21 92 (21 9)	260 3-263 1 (261 7)	0 05-0 37 (0 21)	0 55-0 59 (0 57)	2509 0-2515 0 (2512 0)
Straw	5 98-6 02 (6 0)	68 7-71 3 (70 0)	0 05-0 09 (0 07)	0 15-0 17 (0 16)	400 0-404 0 (402 0)
Grain	4 97-5 03 (5 0)	25 9-28 9 (27 4)	0 01-0 03 (0 02)	0 01-0 03 (0 02)	105 0-107 0 (106 0)

TABLE-7(a)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE > 1200m, msl

GROWTH STAGES	B	Al	V	Cr	Mn
Seedling	32.8-33.15 (33.0)	336.0-338.0 (337.0)	0.34-0.78 (0.56)	1.40-1.80 (1.60)	2633.0-2637.0 (2635.0)
Tillering	22.9-23.10 (23.0)	313.9-318.1 (316.0)	0.27-0.51 (0.39)	1.03-1.14 (1.05)	2555.0-2557.0 (2556.0)
Panicle initiation	19.9-20.05 (20.0)	224.6-227.4 (226.0)	0.13-0.33 (0.23)	0.47-0.67 (0.57)	2436.0-2454.0 (2445.0)
Straw	5.90-6.10 (6.0)	63.6-68.4 (66.0)	0.04-0.05 (0.05)	0.07-0.11 (0.09)	369.0-381.0 (375.0)
Grain	3.91-4.09 (4.0)	9.1-12.1 (10.6)	0.01-0.03 (0.02)	0.04-0.06 (0.05)	89.0-99.0 (94.0)

TABLE-7(b)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE < 700m, msl

GROWTH STAGES	Fe	Co	Ni	Cu	Zn
Seedling	328.4-379.6 (354.0)	0.54-0.84 (0.69)	0.78-1.18 (0.98)	20.1-23.9 (22.0)	10.0-15.0 (12.5)
Tillering	273.1-402.9 (338.0)	0.36-0.46 (0.41)	0.50-0.90 (0.70)	16.7-19.3 (18.0)	7.4-11.0 (9.2)
Panicle initiation	263.2-336.8 (300.0)	0.22-0.38 (0.30)	0.34-0.54 (0.44)	12.4-13.6 (13.0)	5.6-8.6 (7.1)
Straw	153.3-190.7 (172.0)	0.07-0.25 (0.16)	0.36-0.46 (0.41)	1.30-4.70 (3.0)	20.9-23.1 (22.0)
Grain	35.5-64.5 (50.0)	0.02-0.08 (0.05)	0.22-0.34 (0.28)	3.30-4.70 (4.0)	17.2-18.8 (18.0)

ALTITUDE 701-1200m, msl

GROWTH STAGES	Fe	Co	Ni	Cu	Zn
Seedling	326.5-339.3 (332.9)	0.41-0.45 (0.43)	1.15-1.23 (1.19)	22.7-25.3 (24.0)	11.6-14.4 (13.0)
Tillering	307.7-318.7 (313.2)	0.32-0.36 (0.34)	0.69-0.77 (0.73)	18.0-20.0 (19.0)	8.0-12.0 (10.0)
Panicle initiation	276.0-288.0 (282.0)	0.22-0.28 (0.23)	0.36-0.40 (0.38)	13.6-16.4 (15.0)	5.6-8.8 (7.2)
Straw	218.1-231.9 (225.0)	0.08-0.12 (0.10)	0.46-0.50 (0.48)	8.4-11.0 (9.7)	18.0-22.0 (20.0)
Grain	81.7-86.3 (84.0)	0.04-0.06 (0.05)	0.22-0.28 (0.25)	4.5-7.5 (6.0)	8.3-11.5 (9.9)

TABLE-7(b)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE > 1200m, msl

GROWTH STAGES	Fe	Co	Ni	Cu	Zn
Seedling	342.0-420.0 (381.0)	0.52-0.79 (0.69)	0.79-1.09 (0.94)	20.0-22.0 (21.0)	9.8-14.2 (12.0)
Tillering	300.0-394.0 (347.0)	0.42-0.62 (0.52)	0.64-0.84 (0.74)	13.9-18.1 (16.0)	6.8-9.2 (8.0)
Panicle initiation	274.0-362.0 (318.0)	0.25-0.45 (0.35)	0.45-0.54 (0.49)	6.6-9.4 (8.0)	4.0-6.0 (5.0)
Straw	259.0-311.0 (285.0)	0.11-0.20 (0.15)	0.34-0.54 (0.44)	5.2-10.0 (7.6)	19.3-20.7 (20.0)
Grain	115.3-124.7 (120.0)	0.03-0.05 (0.04)	0.16-0.34 (0.25)	3.5-6.5 (5.0)	10.5-11.5 (11.0)

TABLE-7(c)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE < 700m, msl

GROWTH STAGES	Se	Mo	Cd
Seedling	1.19-1.59 (1.39)	0.64-0.84 (0.74)	0.39-0.45 (0.42)
Tillering	0.73-1.13 (0.93)	0.49-0.61 (0.55)	0.26-0.36 (0.31)
Panicle initiation	0.53-0.73 (0.63)	0.31-0.39 (0.35)	0.19-0.27 (0.23)
Straw	0.06-0.07 (0.07)	0.24-0.32 (0.28)	0.031-0.049 (0.04)
Grain	0.029-0.031 (0.03)	0.17-0.21 (0.19)	0.003-0.017 (0.01)

ALTITUDE 701-1200m, msl

GROWTH STAGES	Se	Mo	Cd
Seedling	1.64-1.84 (1.74)	0.50-0.66 (0.58)	0.35-0.41 (0.38)
Tillering	1.03-1.19 (1.11)	0.48-0.60 (0.54)	0.24-0.30 (0.27)
Panicle initiation	0.55-0.59 (0.57)	0.39-0.45 (0.42)	0.16-0.20 (0.18)
Straw	0.15-0.17 (0.16)	0.20-0.24 (0.22)	0.04-0.06 (0.05)
Grain	0.019-0.021 (0.02)	0.13-0.15 (0.14)	0.012-0.028 (0.02)

TABLE-7(c)

CONCENTRATION OF TRACE ELEMENTS (PPM) DETECTED IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE > 1200m, msl

GROWTH STAGES	Se	Mo	Cd
Seedling	1.40-1.80 (1.60)	0.47-0.87 (0.67)	0.26-0.44 (0.35)
Tillering	1.20-1.60 (1.40)	0.44-0.64 (0.54)	0.25-0.41 (0.33)
Panicle initiation	0.47-0.67 (0.57)	0.30-0.48 (0.39)	0.15-0.23 (0.19)
Straw	0.07-0.11 (0.09)	0.24-0.36 (0.30)	0.03-0.05 (0.04)
Grain	0.046-0.054 (0.05)	0.15-0.25 (0.20)	0.013-0.027 (0.02)

TABLE-8a

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS AT SEEDLING STAGE AND ALTITUDE

Trace elements Concentration.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	- 0.13	- 0.57*	0.10
Aluminium	0.02	- 0.19	- 0.95**
Vanadium	0.17	0.54*	0.98**
Chromium	0.30	- 0.92**	0.03
Manganese	- 0.17	- 0.77**	- 0.32
Iron	- 0.17	- 0.01	0.61**
Cobalt	- 0.54*	0.88**	- 0.81**
Nickel	0.05	- 0.67**	- 0.68**
Copper	0.49*	0.60**	0.46*
Zinc	- 0.03	- 0.50*	- 0.24
Selenium	0.07	0.27	0.48*
Molybdenum	- 0.04	0.45*	- 0.55*
Cadmium	0.03	0.18	- 0.07
	$r^2 = 0.676$	$r^2 = 0.956$	$r^2 = 0.981$

* significant at $p < 0.05$, ** significant at $p < 0.01$

TABLE-8b

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS AT TILLERING STAGE AND ALTITUDE

Trace elements Concentration.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	- 0.11	- 0.56*	0.28
Aluminium	0.01	- 0.46*	- 0.19
Vanadium	0.09	0.48*	0.29
Chromium	0.46*	- 0.91**	- 0.15
Manganese	- 0.55*	- 0.78**	- 0.29
Iron	- 0.17	- 0.31	0.45*
Cobalt	0.15	0.85**	- 0.83**
Nickel	- 0.05	- 0.41*	- 0.70**
Copper	0.10	0.28	- 0.15
Zinc	0.06	- 0.18	- 0.25
Selenium	0.16	0.07	0.27
Molybdenum	- 0.13	- 0.64**	- 0.26
Cadmium	0.14	0.01	0.52*
	$r^2 = 0.850$	$r^2 = 0.968$	$r^2 = 0.952$

*significant at $p < 0.05$, ** significant at $p < 0.01$.

TABLE-8c

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS AT PANICLE STAGE AND ALTITUDE

Trace elements Concentration.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	- 0.41*	- 0.49*	- 0.18
Aluminium	0.05	0.01	0.16
Vanadium	0.26	0.40*	- 0.63**
Chromium	0.06	- 0.76**	0.00
Manganese	- 0.73**	- 0.80**	- 0.52*
Iron	0.01	0.08	0.81**
Cobalt	0.24	0.50*	- 0.66**
Nickel	0.27	- 0.05	0.03
Copper	0.08	0.09	0.50*
Zinc	- 0.02	0.15	- 0.32
Selenium	- 0.18	0.05	- 0.15
Molybdenum	0.31	- 0.71**	- 0.74**
Cadmium	- 0.01	0.31	- 0.07
	$r^2 = 0.761$	$r^2 = 0.846$	$r^2 = 0.864$

* significant at $p < 0.05$, **significant at $p < 0.01$

TABLE-8d

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS IN GRAIN AND ALTITUDE

Trace elements Concentration.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	0.16	0.34	0.15
Aluminium	- 0.22	0.12	- 0.05
Vanadium	- 0.40*	0.47*	- 0.18
Chromium	0.06	- 0.17	0.40*
Manganese	- 0.68**	0.52*	0.28
Iron	- 0.42*	0.55*	0.64**
Cobalt	0.15	- 0.01	- 0.69**
Nickel	0.30	0.19	- 0.23
Copper	- 0.53*	0.43*	0.26
Zinc	0.58**	- 0.41*	- 0.15
Selenium	- 0.21	0.06	0.61**
Molybdenum	- 0.22	- 0.31	0.17
Cadmium	0.19	- 0.32	0.41*
	$r^2 = 0.815$	$r^2 = 0.878$	$r^2 = 0.921$

*significant at $p < 0.05$, ** significant at $p < 0.01$

TABLE-8e

CORRELATION BETWEEN CONCENTRATIONS OF TRACE ELEMENTS IN STRAW AND ALTITUDE

Trace elements Concentration.	Altitude		
	<700m,msl	701-1200m,msl	>1200m,msl
Boron	0.27	- 0.25	0.23
Aluminium	- 0.42*	- 0.14	- 0.32
Vanadium	- 0.21	0.49*	0.45*
Chromium	0.21	0.05	0.28
Manganese	- 0.80**	0.97**	- 0.72**
Iron	0.24	0.63**	0.69**
Cobalt	0.21	0.75**	- 0.34**
Nickel	0.01	- 0.34	0.16
Copper	- 0.20	0.64**	0.62**
Zinc	0.28	0.71**	0.22
Selenium	0.25	- 0.65**	- 0.86**
Molybdenum	0.20	0.32	0.20
Cadmium	- 0.20	- 0.40*	0.56**
	$r^2 = 0.964$	$r^2 = 0.973$	$r^2 = 0.938$

* significant at $p < 0.05$, ** significant at $p < 0.01$

TABLE-9a

CORRELATION COEFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE PLANT AT SEEDLING STAGE

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.94	0.11	0.30	0.09	0.28	-0.03	0.22	0.21	0.30	0.11	0.14	-0.14
Al		1	-0.88**	-0.08	0.28	-0.20	0.46*	0.43*	-0.14	-0.04	-0.13	0.23	0.28
V			1	0.18	-0.24	0.36	-0.45*	-0.36	0.19	0.17	0.15	-0.16	-0.21
Cr				1	0.29	0.18	0.02	0.42*	0.30	0.14	0.11	0.30	0.08
Mn					1	0.09	0.11	0.29	0.25	0.09	0.30	0.17	0.48*
Fe						1	-0.20	0.14	0.47*	0.10	0.12	0.19	0.21
Co							1	0.29	-0.14	0.14	0.01	0.52*	0.28
Ni								1	0.06	0.21	0.004	0.36	0.26
Cu									1	0.06	0.36	0.10	0.21
Zn										1	-0.07	0.23	0.07
Se											1	0.06	0.50*
Mo												1	0.45*
Cd													1

*Significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.884$

TABLE - 9b

CORRELATION COEFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE PLANT AT TILLERING STAGE

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.33	0.21	0.29	0.19	0.02	-0.08	0.12	0.15	0.21	0.25	0.44*	0.45*
Al		1	0.26	0.30	0.29	0.26	0.09	0.26	0.47*	0.13	0.18	0.26	0.04
V			1	0.46*	0.26	0.42*	0.28	0.26	0.52*	0.26	0.15	0.03	0.26
Cr				1	0.49*	0.29	0.09	0.47*	0.12	0.22	0.30	0.29	0.15
Mn					1	0.55*	0.25	0.53*	0.29	0.25	0.21	0.28	0.06
Fe						1	0.20	0.33	0.42*	0.006	-0.003	0.13	0.09
Co							1	0.55*	0.17	0.19	0.25	0.14	-0.11
Ni								1	0.18	0.29	0.22	0.31	0.06
Cu									1	0.18	0.002	0.10	0.06
Zn										1	0.25	0.22	0.02
Se											1	0.23	0.19
Mo												1	0.33
Cd													1

*significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.756$

TABLE - 9c

CORRELATION COEFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE PLANT AT PANICLE STAGE

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.21	0.17	0.31	0.29	0.08	0.14	0.25	0.15	0.03	0.25	0.23	0.02
Al		1	0.25	0.15	0.26	0.47*	0.23	0.09	0.28	0.17	0.26	0.20	0.26
V			1	0.23	0.30	0.56**	0.56**	0.53*	0.32	0.11	0.42*	0.32	0.51*
Cr				1	0.20	0.25	0.17	0.30	0.06	-0.07	0.35	0.34	0.27
Mn					1	0.57**	0.17	0.05	0.25	0.16	0.49*	0.17	0.40*
Fe						1	0.51*	0.30	0.33	0.16	0.56**	0.14	0.55*
Co							1	0.65**	0.26	-0.05	0.35	0.36	0.63**
Ni								1	0.21	-0.01	0.35	0.47*	0.49*
Cu									1	0.07	0.30	0.18	0.20
Zn										1	0.09	0.01	0.01
Se											1	0.31	0.41*
Mo												1	0.30
Cd													1

*Significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.890$

TABLE - 9d

CORRELATION COEFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE PLANT IN GRAIN

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.20	0.44*	0.21	0.31	0.10	0.11	0.13	0.35	-0.06	0.31	0.18	0.38
Al		1	0.25	0.41*	0.35	-0.08	0.19	0.44*	0.18	0.19	0.30	0.47*	0.16
V			1	-0.02	0.08	0.49*	0.27	0.19	0.21	0.19	0.03	0.16	-0.04
Cr				1	0.34	-0.47*	-0.24	0.11	0.18	-0.05	0.45*	0.43*	0.55*
Mn					1	-0.32	-0.14	0.16	0.48*	-0.36	0.48*	0.05	0.18
Fe						1	0.53*	0.09	-0.17	0.34	-0.47*	-0.08	-0.35
Co							1	0.27	-0.17	0.30	-0.32	-0.01	-0.18
Ni								1	0.05	0.40*	0.16	0.17	-0.01
Cu									1	-0.16	0.28	0.12	0.19
Zn										1	0.03	0.25	0.09
Se											1	0.24	0.49*
Mo												1	0.18
Cd													1

*Significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.871$

TABLE - 9e

CORRELATION COEFFICIENT OF THE CONCENTRATIONS OF VARIOUS ELEMENTS DETECTED IN RICE PLANT IN STRAW

	B	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Mo	Cd
B	1	0.25	0.34	0.24	0.17	0.07	-0.04	0.34	0.26	0.15	0.10	0.28	0.53*
Al		1	0.47*	0.02	0.41*	0.25	0.24	0.33	0.41*	0.05	0.14	0.08	0.13
V			1	0.16	0.53*	0.19	0.22	0.34	0.47*	-0.13	0.13	0.22	0.29
Cr				1	-0.06	0.33	0.03	0.16	0.11	0.18	0.23	0.51*	0.34
Mn					1	-0.02	0.15	0.20	0.43*	-0.41*	0.04	-0.05	0.19
Fe						1	0.33	0.20	0.12	0.34	0.28	0.32	0.08
Co							1	0.05	0.02	0.33	-0.23	0.23	0.04
Ni								1	0.42*	0.26	0.22	0.49*	0.43*
Cu									1	-0.12	0.49*	0.02	0.08
Zn										1	0.12	0.39	0.11
Se											1	0.33	-0.03
Mo												1	0.43*
Cd													1

*Significant at $p < 0.05$, **significant at $p < 0.01$, $r^2 = 0.958$

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CHAPTER-V

ENZYME ACTIVITY- A POSSIBLE INDICATOR FOR TOXICITY/ - DEFICIENCY OF TRACE ELEMENTS IN RICE PLANT

5.1 INTRODUCTION

Soil test and plant analysis were used as tools for diagnosing the deficiency/toxicity of trace elements in rice soils and plants at different growth stages as described earlier. In many enzymes, these trace elements are their constituents. The physiological significance of trace elements has been manifested by the role played by the enzymes. Hence, it transforms the science of trace elements into an active biological discipline Trace elements are involved in almost all known processes in which substances are synthesized and transformed with the aid of enzymes. A metal in an enzyme affects not only the enzyme activity, but also the specificity of its actions. The same metal can perform several functions in the same enzyme. The special role of the metal in the enhancement of an enzyme catalytic activity is determined by the binding between the metal ion and the protein moiety of the enzymes. The function of the metal is to establish and stabilise the intermediate linkage in an organo-metal complex. A metal may also serve as a bridge between enzymes and substrates [1]. Some Workers have reported that to assess the mineral nutrient status of plants, the enzymatic method involving marker enzymes may be one of the approaches. The enzymatic methods are based on the fact that depending on the mineral nutrient status of the plant, the activity of certain enzymes is lower or higher in the tissue [2]. Guided by the report of previous workers, we have carried out the estimation of the enzyme activity to throw light on the concentration of the trace elements in the rice plant at different growth stages.

In the previous chapter, Iron was analysed to be present in excess amount while Zinc was found to be deficient in the rice plant at all growth stages. Both these elements have important roles in the growth and development of the plant which may ultimately contribute to the low yield. Therefore, the two enzymes selected for this study were **Catalase** and **Alcohol Dehydrogenase (ADH)** in which **Iron** and **Zinc** are respectively involved. Iron is an element which acts as a catalyst for the synthesis and maintenance of chlorophyll and also an essential component of many enzymes of which catalase is one of them. The enzyme catalase is widely distributed in nature and is found in all aerobic micro-organism as well as in plant and animal cells. Most catalases contain the protophyrin IX prosthetic group [3, 4, 5] as shown in **FIG-14.** Each group consists of four submits of almost 60,000 Dal each giving a protein of approximately 240,000 Dal [4, 6]. Each subunit contains iron atoms per protein of which iron is an important ingredient of catalase. Catalase is reported to show a maximal activity at pH 6.5 to 8.5 but it is stable only at alkaline pHs [6]. Hydrogen peroxide formed by superoxide dimutase and by the flavin-linked oxidises [7] is decomposed by the heme enzyme catalase as shown in the reaction below

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (1)$$

The quantity of oxygen produced by one milligram of the enzyme catalase from H_2O_2 decomposition is about 2740 litre/hr at 0°C [8]. Moreover, in the presence of catalase, the activation energy required for the decomposition of H_2O_2 is only 6,400 cal/mole. But in its absence, the energy required is 18,000 cal/mole [9]. As catalase accelerates the decomposition of hydrogen peroxide, it prevents its accumulation. This protects the cell decomposition as Hydrogen peroxide is a toxic substance [4].

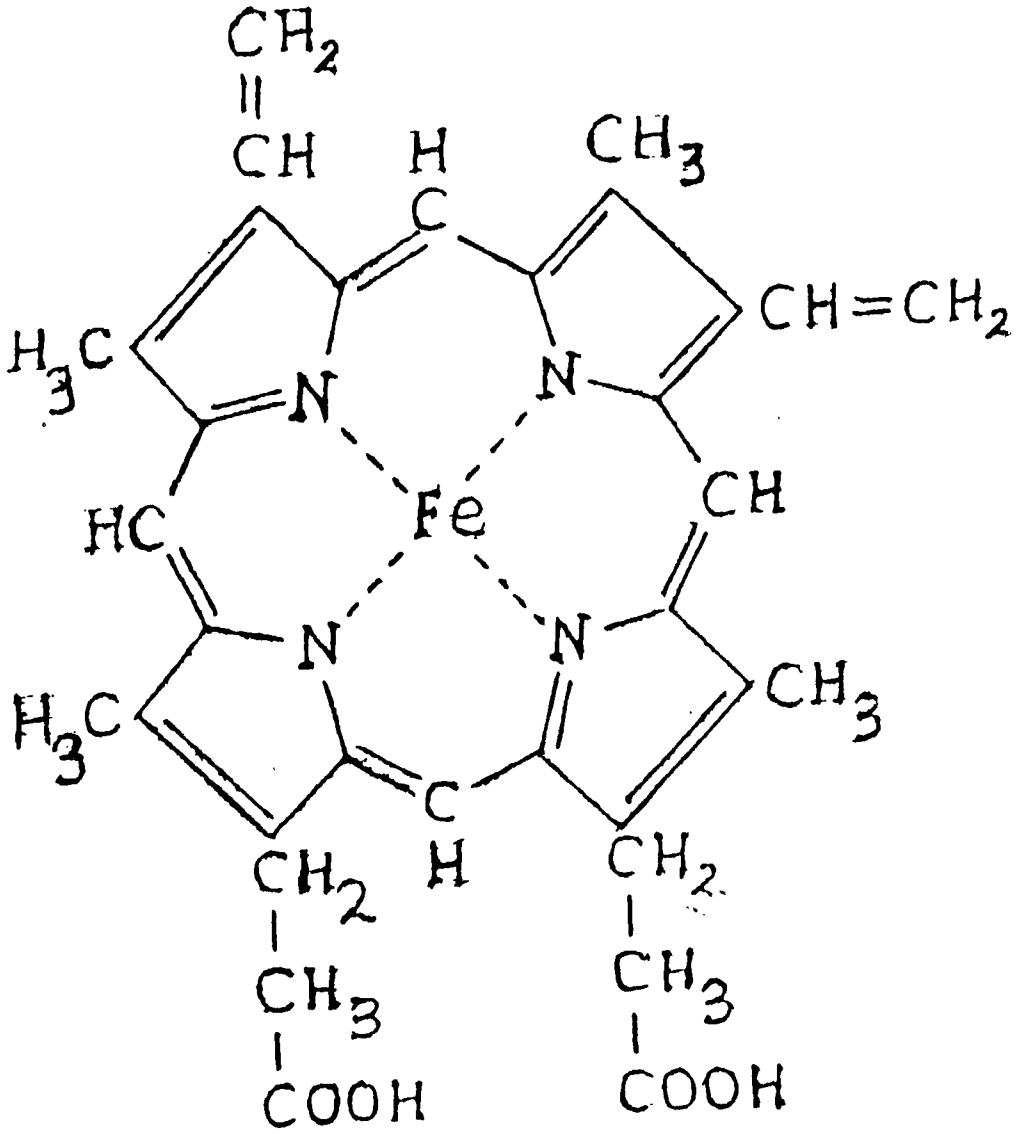


FIG-14. PROTOPORPHYRIN IX

Zinc is an integral component of many enzymes, indispensable to their catalytic functions and structural stability. Most recently, zinc enzymes have been shown to monitor the replication, transcription and translation of the genetic materials of all species accounting for the absolute dependence of all forms of life on this element [10]. It is also involved in the biosynthesis of many plant hormones and is a component of a variety of enzymes in which Alcohol dehydrogenase (ADH) is one of them. This enzyme requires Zn as a cofactor [7] as shown in **FIG-15**. It has a molecular weight of 79,870 and is composed of two similar subunits [11]. Each subunit contains two atoms of Zinc. This enzyme is reported to be stable at pH 6.5 to 7.0 [12]. ADH catalyses the reduction of acetaldehyde to ethanol in alcoholic fermentation.

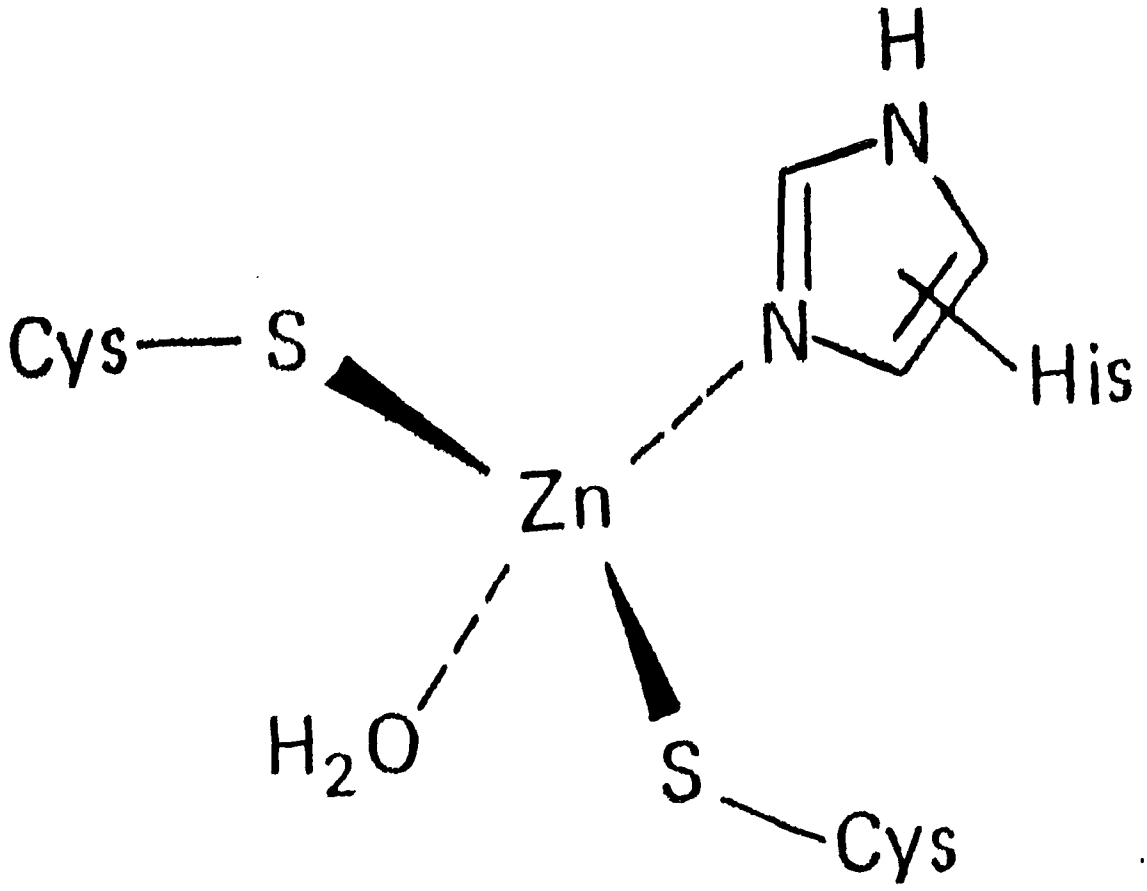
$$C_2H_5OH + NAD \xrightarrow{ADH} CH_3CHO + NADH + H \qquad (2)$$

The importance of dehydrogenases is their ability to either use molecular oxygen directly as hydrogen acceptors (aerobic dehydrogenases) or to operate through other hydrogen acceptor (anaerobic dehydrogenases).

5.2 EXTRACTION OF CATALASE AND ALCOHOL DEHYDROGENASE

Rice plant tissues were collected at different growth stages, namely seedling, tillering and panicle at the same time and from the same areas as those used for soils and rice plant analysis. Plant samples were washed in tap water and then rinsed with triple distilled water. They were stored at -80° C and brought to room temperature, 24 hrs before use.

For the extraction of catalase, about 0.20g of the green tissue was ground in a chilled mortar and pestle by using cold 0.1M phosphate buffer (pH 7.0). The brei was clarified by centrifugation at 7000 rpm for 10-15 minutes [13].



FIG–15 STRUCTURE OF ALCOHOL DEHYDROGENASE

To extract ADH, the same amount of the green tissue was ground in a chilled mortar and pestle by using 2 ml of 0.15M Tris-HCl buffer (pH 8.0) containing 10 mM b-mercaptoethanol. The brei was clarified by centrifugation and the aliquots of the supernatant was taken for the assay of ADH as described by Hanson et al [14].

5.3 QUANTITATIVE ASSAY OF CATALASE AND ALCOHOL DEHYDROGENASE

The above supernatant collected was assayed for catalase using the approach of Boismenic et al [4]. The Molar extinction coefficient of H_2O_2 at 240nm is 0.040 cm^2 /mmole [15]. Catalase activity can be measured by following either the decomposition of H_2O_2 or the liberation of O_2. For our work, we measured the decomposition of H_2O_2.

In our measurement, catalase activity was monitored at different time intervals ranging from 0 seconds to 90 seconds at 240nm wavelength at a step of 5 seconds. Our experiment showed that for an interval of 60 seconds, the absorbance became minimum. This happened because the decomposition of hydrogen peroxide was maximum [**FIG-16**] Hence we consider 60 seconds as the time interval required to have the maximum activity of catalase. Similarly, catalase was assayed at different temperatures ranging from 15° C to 30° C at 240nm wavelength at a step of 1° C. The maximum activity obtained was at 25° C [**FIG-17**].

The other supernatant obtained was assayed for ADH using the approach as described by Hanson et al [14]. The molar extinction coefficient of ADH at 340nm is 6.22 cm^2 /mmole [16]. In the reaction (equation 2), the absorption of light by the NADH produced was maximum at 340nm wavelength. The increase in absorption is proportional to the enzyme activity. ADH activity was first monitored with time in the same way as catalase but at 340nm wavelength. In this case, it was noticed that absorbance increased with increase of time till a particular interval was reached. The

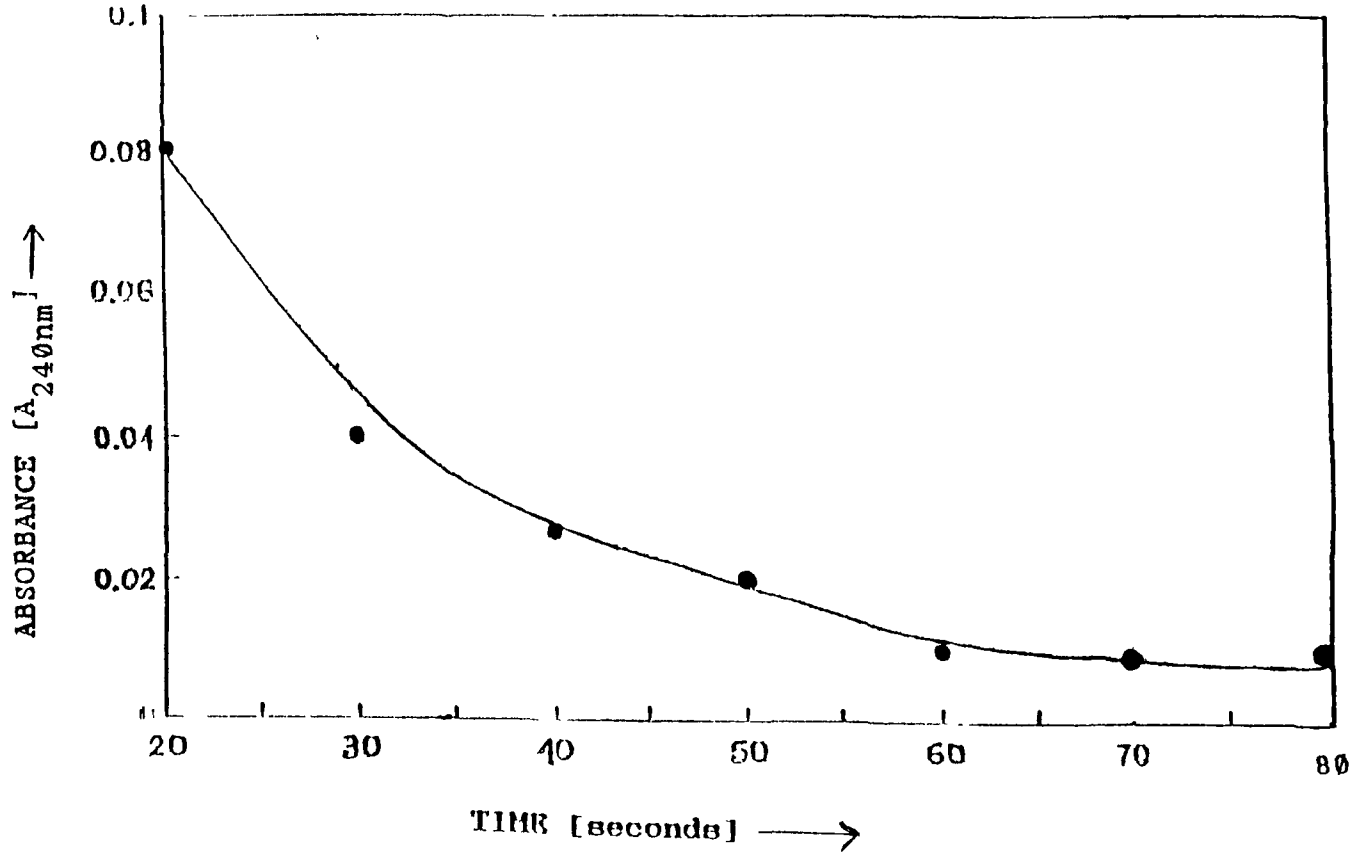


FIG-16. KINETICS OF CATALASE REACTION

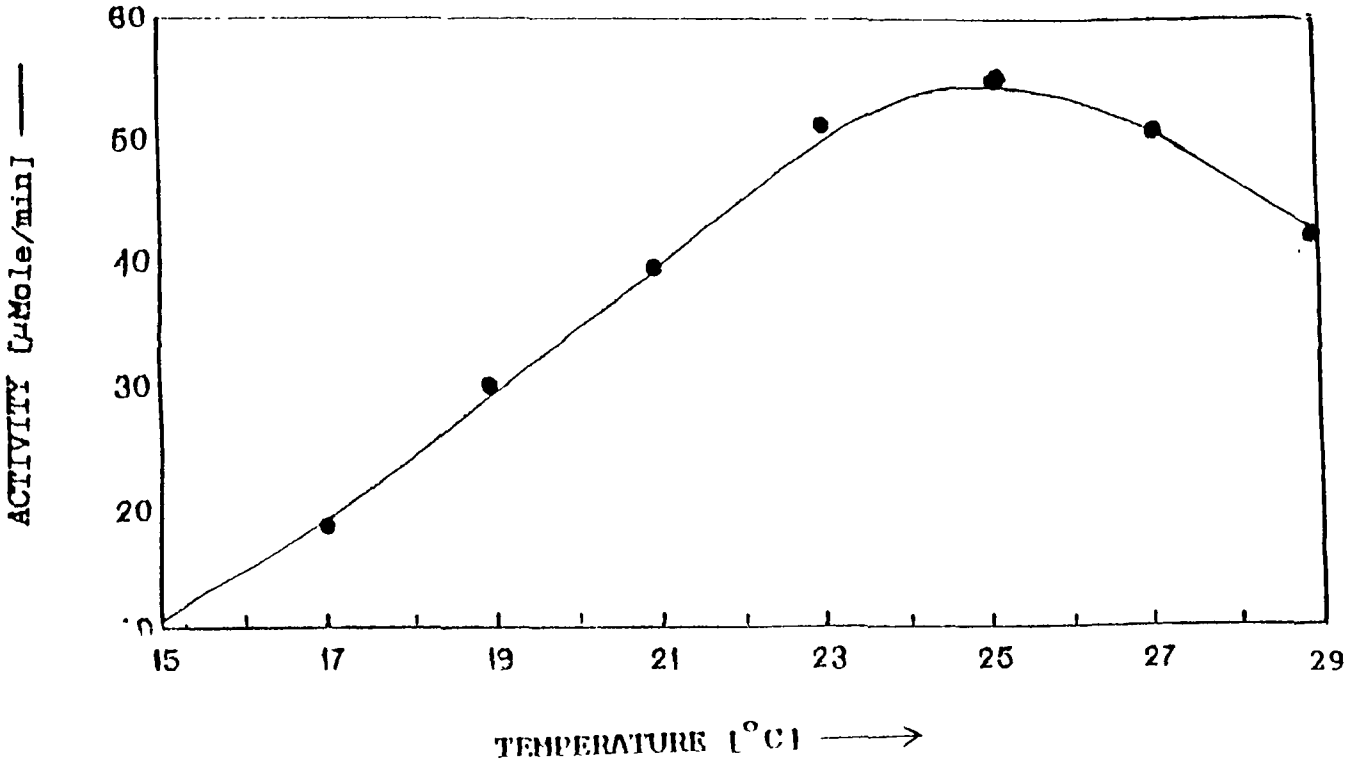


FIG-17. EFFECT OF TEMPERATURE ON CATALASE ACTIVITY

absorbance was found to be maximum for an interval of 60 seconds [**FIG-18**]. This implied that the time required to monitor for maximum ADH activity was 60 seconds. With respect to temperature, ADH was assayed at different temperatures ranging from 15° C to 30° C at 340nm wavelength. The temperature was increased in increments of 1° C. The maximum activity obtained was at 25° C [**FIG-19**].

To measure the activity of the enzymes extracted, the instrument **HITACHI U-2000 spectrophotometer** was used for taking absorbance readings of both the assays. All measurements were repeated at least 5x and the results presented are the mean values and standard deviations of these measurements. The calculation of enzyme activity was given below

$$\text{Enzyme activity} = \frac{1}{\Delta E} \times \frac{1}{\varepsilon} \times \frac{\text{Total volume of the solution}}{\text{Volume of enzyme solution}}$$

Where,

ΔE = change in absorbance

ε = Molar extinction coefficient

5.4 DETERMINATION OF IRON AND ZINC CONCENTRATION

The concentrations of Iron and Zinc were estimated by the same method described in the previous chapter in Distribution of Trace Elements in Rice Plant at different growth stages.

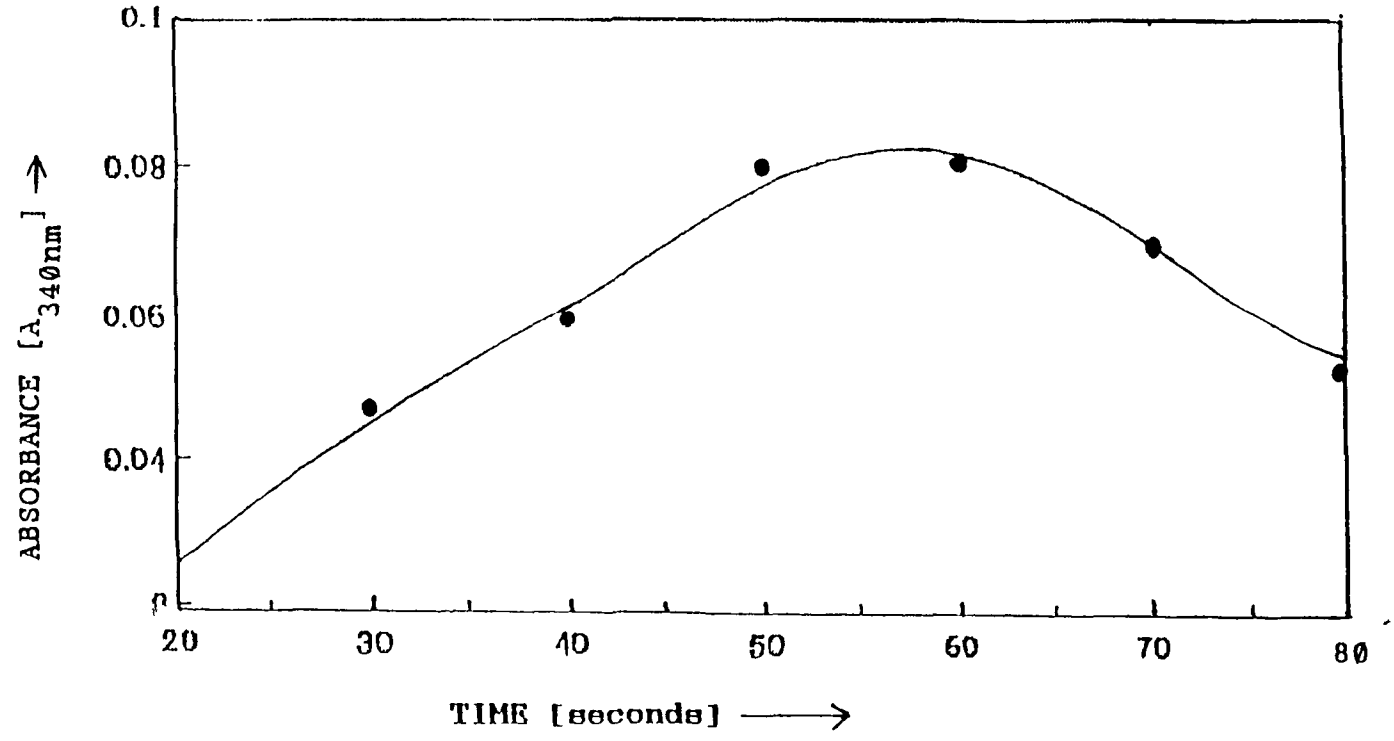


FIG-18. KINETICS OF ADH REACTION

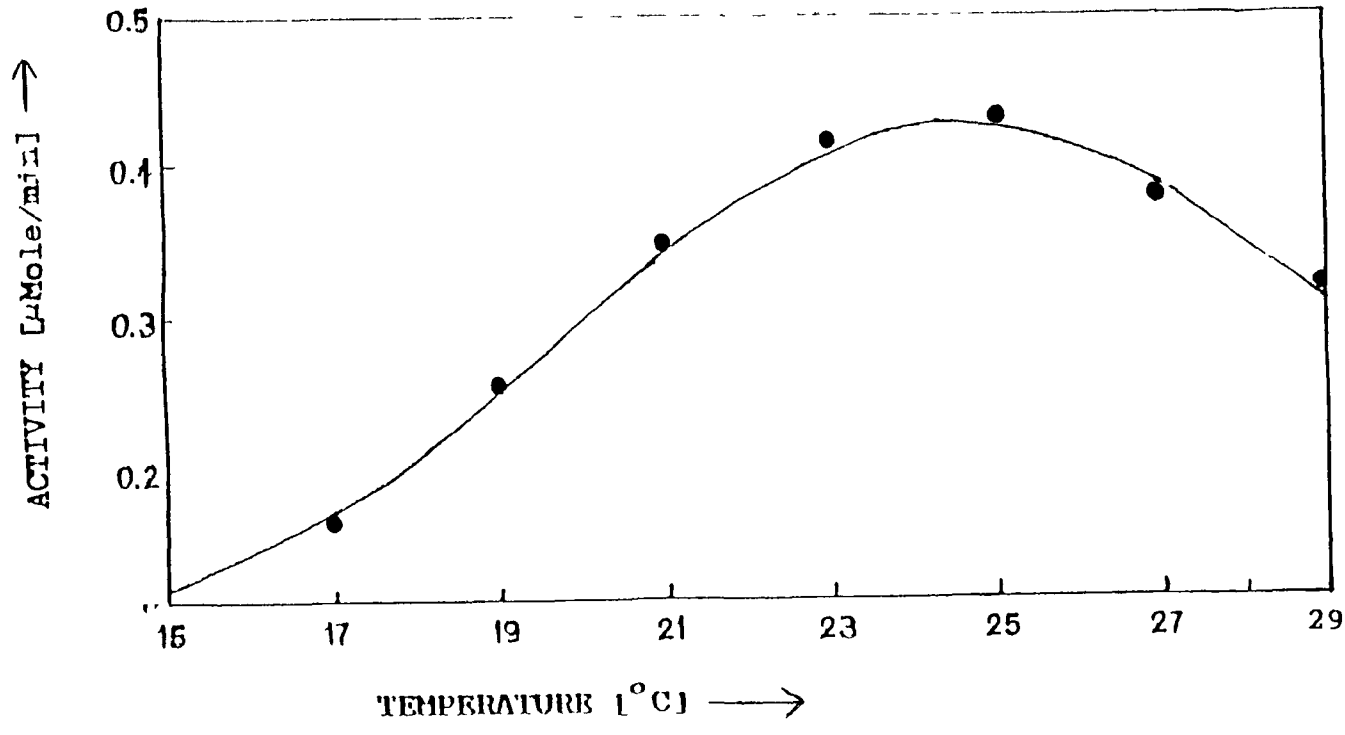


FIG-19. EFFECT OF TEMPERATURE ON ADH ACTIVITY

5.5 RESULTS AND DISCUSSION

Table-11 gives the correlation coefficient of the contents of Iron and Zinc with their enzyme activity, Catalase and ADH as well as with the shoot weight.

It is observed that the Catalase activity is significantly correlated with the Iron concentration as well as with the shoot weight both at the seedling and tillering stages except at the panicle stage. The ADH activity correlates positively with Zinc content, Catalase activity and Iron concentration at panicle stage, with Iron concentration at seedling stage also. While it shows a negative correlation with shoot weight at tillering stage. Similarly, Zinc content shows a positive correlation with Iron concentration and shoot weight at tillering stage. While Iron content gives a positive correlation with shoot weight only at seedling stage.

Iron and Zinc concentration has positive effect on Catalase and ADH activity, respectively. The Iron concentration also has positive effect on ADH activity at seedling stage but it shows a negative effect at panicle stage.

The total concentration of Iron and Zinc at different growth stages and the activities of their corresponding metalloenzymes, Catalase and Alcohol dehydrogenase are given in TABLE-10. The detail analysis of Iron and Zinc had been described in Chapter-IV. The concentration of Iron is present in good amount while the concentration of Zinc is deficient at all growth stages. The probable causes for the Iron toxicity and Zinc deficiency are already mentioned in the earlier chapter. The deficiency of Zinc in the rice plant of Meghalaya may also be due to the high accumulation of Iron.

The activity of catalase was assayed at different growth stages of the rice plant, namely seedling, tillering, and panicle. The activity of catalase is found to decrease from seedling to the panicle stage. This is similar to the trend found for Iron content. Hence, it establishes a relationship between the Iron content and the activity of the

enzyme it involves. It therefore explains that Iron is an important ingredient of the enzyme catalase, enhancing its activity by the binding between the metal ion with the protein moity of the enzymes. According to Gorkin [17], this element can perform several functions in an enzyme such as catalytic process and stabilising the tertiary or quaternary structure of the enzyme. The activity of ADH is also assayed at different growth stages of the rice plant. The activity of ADH decreases with respect to the age of the plant as well as the concentration of Zinc. This shows that Zinc which is a cofactor of ADH enhances its activity by binding NAD with its four atoms to the enzyme. Hence, the plant age attributes to both the ADH activity as well as the Zinc concentration as seen in the case of catalase and Iron.

Correlation study (TABLE-11) shows that Iron and Zinc concentrations have positive effect on Catalase and ADH activity, respectively. The Iron concentration also has positive effect on ADH activity at seedling stage but it shows a negative effect at panicle stage.

Our findings exhibit that the concentrations of Iron and Zinc in the rice plant at different growth stages are related to the activities of their corresponding enzymes. Previous workers have plotted the root ADH activity as a function of Zinc concentration which indicates that Zinc content of the plant does indeed influence the ADH activity [18]. This supports our finding.

CONCLUSION:-

From the knowledge of activity of the enzyme, the information of the involved element content can be obtained to have an idea about its toxicity or deficiency in the plant. Hence, estimating the enzyme activity can be adopted as one of the methods for diagnosing the deficiency or excess amount of the trace element. On the other hand, study on the influence of trace elements on enzyme activity in various crops is vital for assessing the functional or active portion of the trace element and in explaining the causes for the nutritional disorder.

TABLE-10

TRACE ELEMENTS CONTENT AND ENZYME ACTIVITY IN RICE PLANT AT DIFFERENT GROWTH STAGES

ALTITUDE < 700 m, msl

Growth stages	Rice shoot weight (g)	Fe ppm	Catalase activity µmol/min/g F w	Zn ppm	ADH activity µmol/min/g F w
Seedling	0 37-0 43 (0 40)	328 0-380 0 (354 0)	0 176-0 188 (0 182)	10 0-13 0 (12 5)	0 356-0 456 (0 406)
Tillering	9 97-10 39 (10 18)	273 0-403 0 (338 0)	0 118-0 126 (0 122)	7 4-11 0 (9 2)	0 330-0 390 (0 360)
Panicle initiation	13 74-14 36 (14 05)	220 0-294 0 (257 0)	0 066-0 076 (0 071)	5 6-8 6 (7 1)	0 230-0 25 (0 240)

ALTITUDE 701-1200m, msl

Growth stages	Rice shoot weight (g)	Fe ppm	Catalase activity µmol/min/g F w	Zn ppm	ADH activity µmol/min/g F w
Seedling	0 34-0 36 (0 35)	326 5-339 3 (332 9)	0 138-0 142 (0 140)	11 6-14 4 (13 0)	0 522-0 562 (0 542)
Tillering	8 94-10 10 (9 54)	307 7-318 0 (313 2)	0 093-0 097 (0 095)	8 0-12 0 (10 0)	0 407-0 427 (0 417)
Panicle initiation	11 9-14 62 (13 28)	276 0-288 0 (282 0)	0 057-0 061 (0 059)	5 6-8 8 (7 2)	0 295-0 315 (0 305)

ALTITUDE > 1200m, msl

Growth stages	Rice shoot weight (g)	Fe ppm	Catalase activity µmol/min/g F w	Zn ppm	ADH activity µmol/min/g F w
Seedling	0 31-0 33 (0 32)	342 0-420 0 (381 0)	0 160-0 168 (0 164)	9 8-14 2 (12 0)	0 425-0 485 (0 455)
Tillering	8 65-9 25 (8 95)	300 0-394 0 (347 0)	0 136-0 144 (0 140)	6 8-9 2 (8 0)	0 375-0 395 (0 385)
Panicle initiation	12 63-13 33 (12 98)	274 0-362 0 (318 0)	0 087-0 095 (0 091)	4 0-6 0 (5 0)	0 253-0 267 (0 260)

TABLE-11

CORRELATION COEFFICIENT OF THE TRACE ELEMENTS (Fe AND Zn) AND ENZYME ACTIVITY (CATALASE AND ADH) IN RICE PLANT AT DIFFERENT GROWTH STAGES

	Growth stages	Iron Concentration.	Catalase activity	Zinc Concentration.	ADH activity
Rice shoot weight	(a) Seedling	0.54*	0.56**	- 0.16	- 0.06
	(b) Tillering	0.10	0.30*	0.33*	- 0.45*
	(c) Panicle	0. 14	0.09	- 0.21	0.08
Iron Concentration	(a) Seedling		0.67**	0.05	0.31*
	(b) Tillering		0.30*	0.35*	0.17
	(c) Panicle		0.18	- 0.07	- 0.40*
Catalase activity	(a) Seedling			0.01	0.16
	(b) Tillering			- 0.16	0.24
	(c) Panicle			0.08	0.31*
Zinc Concentration	(a) Seedling				0.55*
	(b) Tillering				0.42*
	(c) Panicle				0.35*

*Significant at $p < 0.05$, **significant at $p < 0.01$.

r^2 at Seedling = 0. 523, Tillering = 0. 582, Panicle = 0.534.

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CHAPTER-VI

CONCLUSION

The levels of the concentrations of trace elements can have adverse or beneficial effects in soils, plants and animals. As a result, studies on trace elements in such systems have brought together Scientists of many disiplines such as Physics, Chemistry and Biochemistry. Their essentiality for living systems has been well recognised. Our study dealt the problem of estimating of thirteen trace elements in rice soils and their distribution in plants at different growth stages using different spectroscopic techniques.

Rice is the main crop of the people of Meghalaya but its production is low. Perhaps, lack of information about the status of trace elements in rice soils and plant is one of the reasons why the farmers fail to improve the yield. Previous workers have only studied for a very few elements like Mn, Cu and Fe in the soil and not in the plants. We studied a broader spectrum of elements, thirteen in all, both in the soil and plants at different growth stages. Before the analysis of the soil for these elements, some of the physico-chemical properties of the soil such as pH, organic carbon, CEC and texture have also been studied to have an idea about the nature of the soil which consequently affect the presence of these elements.

Our investigation shows that the soil of Meghalaya are acidic in nature and are fairly rich in organic matter as well as the CEC. The clay content varies from 11.9 to 43.5 percent. Soil analysis shows that most of the rice growing areas have low content of Boron, Zinc, Cobalt, Chromium, Nickel, Selenium and Cadmium but adequate amount of Vanadium, Iron, Manganese, Aluminium, Copper and Molybdenum. The imbalanced distribution of the trace elements in the soils might be one of the factors which contributed to the low yield. Hence our

results clearly indicate the need for a closer monitoring of important elements like Zinc, Boron, Cobalt, Iron, Manganese and Aluminium in acid alfisols of Meghalaya under rice cultivation, besides the major plant nutrients to enhance rice production in the state.

Correlation studies confirm that the altitude does have significant effect on the content of some of the trace elements. The elements like Boron, Copper, Zinc and Manganese, their concentrations are found to be more at the lower altitude while the element Aluminium at higher altitude. The concentrations of Vanadium, Chromium, Iron, Cobalt, Nickel, Selenium, Molybdenum and Cadmium show no variation with altitudes.

In soil, Zinc is highly correlated with Copper which implies that there is synergestic effect between them and their occurance might be similar. Iron also shows same relationship with Copper but not so significant. The positive correlation of Zinc with other elements like Iron and Manganese is observed but negative with Aluminium. The element Aluminium shows a correlation with Manganese which suggests the antagonistic effect between them.

Analysis of plants at different growth stages shows that the concentrations of the trace elements i.e. Aluminium, Manganese and Iron are higher than the critical limits. We suspect that this causes toxicity to the rice plant especially at the tillering stage. The concentrations of Boron, Vanadium, Nickel, Chromium, Copper, Molybdenum and Cobalt is considered to be adequate for the normal growth of the plant. Zinc is considered to be deficient in the rice plant as the concentration of this element is found to be deficient in all the growth stages.

The contents of the trace elements in the plant show variation with altitude. The concentrations of the elements Vanadium, Chromium, Cobalt and Copper are effected by altitude. At mid-altitude, Vanadium, Cobalt and Copper

show a positive correlation almost at all growth stages while Chromium gives a negative correlation. Boron and Aluminium gives a negative correlation with low and mid-altitude especially at tillering stage. The elements Selenium and Cadmium show that their concentrations tended to be more at some growth stages especially in grain at higher altitudes. The concentrations of Manganese and Nickel decrease with increase of altitude at the initial growth stages. For elements Molybdenum and Zinc, a negative correlation is observed with mid-altitude though no variation is shown in grain and straw by Molybdenum. The element Zinc shows a positive correlation in straw at mid-altitude and in grain at low altitude. The element Iron is found to be more at higher altitude at the initial stages namely seedling and tillering but shows a negative correlation at the latter stages like panicle and grain. In straw, it gives a positive correlation with mid-altitude only.

Correlation studies shows that Iron has positive correlations with many elements especially at the panicle stage. This indicates that the content of this element enhances more the uptake of other trace elements in the initial stages compared to the later one. Enhanced uptake of Iron by the plant might have then affect the uptake of other trace elements necessary for other metabolic functions.

The elements Manganese and Copper correlate with other elements in the latter growth stages of the plant. With Vanadium, Copper and Aluminium, Manganese correlates positively in straw and in grain with Copper and Selenium. Similarly Copper correlates with elements Aluminium, Vanadium, Nickel and Selenium in straw. A positive correlation is also observed in the tillering stage with Aluminium, Vanadium and Iron. The element Zinc is found to correlate only

with two elements that is with Nickel in grain while with Manganese it gives a negative correlation in straw.

Vanadium correlates with most of the elements. At the seedling stage, it shows a negative correlation with Cobalt and Aluminium. The negative correlation with Aluminium might have resulted due to the pH of the soil which favours the availability of the element Aluminium. Like Vanadium, Nickel also correlates with many elements. The positive correlation of Nickel with Cobalt at the tillering and panicle stagesis expected as they are associated geochemically and hence have similar physiological role.

Summarizing our analysis, we come to the conclusion that the three elements Iron, Aluminium and anganese are present in adequate amounts in the soils as well as in the plant especially at the tillering stage. This might have caused toxicity to the plant which ultimately affects the yield. On the other hand, Zinc is found to be deficient in the soil as well as in the plant. Other elements required for the growth and function of the plant like Copper, Cobalt and Molybdenum are considered to be sufficient for the plant.

As the biochemical functions of trace elements are involved in specific enzymes, measurement of the activity of the enzymes in which they are involved helps one to infer the trace elements deficiency or excessive amounts. Catalase and ADH in which Iron and Zinc are respectively involved were selected for activity measurement. The reason for selecting the two enzymes was that Iron was one of the elements present in excess amount while Zinc was deficient both in the rice soil and plant.

The activities of both Catalase and ADH in rice plant are found to decrease from seedling stage to panicle, a trend similar to the content of Iron and Zinc. The correlation study shows that Iron and Zinc concentrations have positive effect on

Catalase and ADH activity respectively. The Iron content also has positive effect on ADH activity at seedling stage but it shows a negative effect at panicle stage. Estimating the enzyme activity can be adopted as one of the diagnostic tool to study the deficiency or excessive amount of trace elements in the plant.

We believe that our study has established the status of a good number of trace elements in the rice soils and their distribution in the plant at different growth stages of Meghalaya state using spectroscopic techniques. Results suggest the need for a closer monitoring of nutrients especially Zinc in acid Alfisols soils of Meghalaya under rice cultivation. for enhancing rice production in the state. The knowledge of the distribution of trace elements in plant serve as the basic information for the future researchers to select the elements for controlled study and in establishing their critical limits.

APPENDIX-I

DISCOVERY OF TRACE ELEMENT REQUIREMENTS

1. **Iron** 17th century
2. **Iodine** 1850 Chatin, A.
3. **Copper** 1928 Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A.
4. **Manganese** 1931 Kemmerer, A. R. and Todd, W. R.
5. **Zinc** 1934 Todd, W. R., Elvehjem, C. A., and Hart, E. B.
6. **Cobalt** 1935 Underwood, E. J., Filmer, J. F., Marston, R., and Lines, E. W.
7. **Molybdenum** 1953 deRenzo, E. C., Kaleita, E., Heytler, P., Oleson, J., Hutchings, B. L., Williams, J. H., Richter, D. A. and Westerfield, W. W.
8. **Selenium** 1957 Schwartz, K. and Foltz, C. M.
9. **Chromium** 1959 Schwartz, K. and Mertz, W.
10. **Tin** 1970 Schwartz, K., Miline, D. R., and Vinyard, E.
11. **Vanadium** 1971 Schwartz, K. Miline, D. B., Hopkins, L. L. and Mohr, H. E.
12. **Fluorine** 1972 Schwartz, K. and Miline, D. B.
13. **Silicon** 1972 Schwartz, K. and Miline, D. B., and Carlisle, E. M.
14. **Nickel** 1973 Nielsen, F. H.

APPENDIX-II

The trace elements B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo and Cd have important role and functions in the plant as well as in human beings. Trace elements like V, Cr, Se and Cd, their detection are also important because their high concentration in plant can lead to dreadful diseases to human being by consuming them. The role and functions of the trace elements are described below:-

BORON: This element is very essential for the plant. It is involved in the nitrogen and protein metabolism and especially Calcium metabolism. It is also involved in the synthesis of pectin, in maintaining the correct water relations with the plant, in the resynthesis of Adenosine triphosphate (ATP), in the translocation of Sugar, in the fruiting processes, in the growth of pollen tube, and in the development of the flowering and fruiting stages. It acts as a catalyst in the plant system [2, 3].

ALUMINIUM: It is one of the major constraints that limits rice plant growth and mineral uptake, thereby decreasing yield. It reduces the transport of many essential elements [4] and replaces element like Calcium which results in increasing the soil pH. However, this element is beneficial for some plants as it is used as a soil acidulant to create an acid soil environment for acid loving plants such as azaleas, rhododendrons, and mountain laurel. It also helps in promoting root growth and acts as an activator of some enzymes though it is not a constituent of true metallo-enzymes [3].

VANADIUM: This element often improves plant yield and has effects on physiological processes such as in stimulating photosynthesis [3, 5]. This element is found to stimulate tiller development and increase the yield of straw and grain.

Vanadium is reported that it is capable of replacing Molybdenum as a catalyst in the process of molecular Nitrogen fixation in a number of soil micro-organism [6].

CHROMIUM: The reports of beneficial effect of Cr on plants are not known. However, the deficiency of this element affects the metabolism of glucose, lipid and protein. It is also a cofactor with insulin. Numerous reports on its toxic effects in man and animals are available. Chronic exposure to high Cr levels have been correlated with lung cancer in man and liver and kidney damage in animals.[3]

MANGANESE: Manganese plays an important role in Nitrogen and Inorganic acid metabolism, carbon dioxide assimilation, Carbohydrates breakdown, formation of Carotene, Riboflavin and Ascorbic acid [7]. In rice, this element is required in photosynthesis and in the oxidation-reduction processes. Several enzymes, such as oxidase, peroxidase, dehydrogenase, decarboxylase, and kinase are activated by Mn [2].

IRON: Iron is an essential element in the Chlorophyll formation but not a constituent of it [2]. It activates a number of oxidases, notably those involving molecular oxygen, but the majority of the Fe enzymes are metalloenzymes and also a constituent of certain proteins [1, 7]. Although it is an essential plant nutrient, involved in promoting rice root development at low concentrations, excess of water soluble Fe in soils retards rice growth [8]. It acts as an inhibitor of the absorption of K by the plant [2].

COBALT: This element is essential for the symbiotic fixation of Nitrogen. It is also the component of vitamin which is needed for the formation of a type of haemoglobin in Nitrogen-fixing nodule tissue [7]. Few enzymes such as acid phosphomonoesterase, aminopolypeptidase, glyceralglycine-peptidase and

argininedesim-idase are activated by Co [1]. Salts of Co such as acetate, chloride and sulphates are highly toxic to human beings.

NICKEL: This element has a positive influence on the activity of an important enzyme known as Nitrate reductase [7]. It is an essential trace element widely distributed in very low concentration in both plant and animal tissues. It is consistently present in ribonucleic acid (RNA) from diverse sources in concentrations many times higher than in the material from which the RNA is isolated [3]. It has been reported that excess of Ni depresses catalase activity in mature plants. Hence it is regarded as a very toxic element which interferes with Fe nutrition or produces other specific signs of toxicity [6]. For human beings, exposure to air borne Ni dust and vapours may cause lung cancer and sinus disorders of the respiratory systems and dermatitis [3].

COPPER: It is a component of metalloenzymes and a regulator of enzymatic actions. At least thirty proteins and enzymes containing Copper have been reported. An important feature of Cu enzymes is their ability to use molecular oxygen directly [3]. Moreover, its importance is indicated in chlorophyll synthesis, carbohydrates and protein metabolism. It also acts as a catalyst for respiration [7]. Though Cu is a very useful element, but copper toxicity is harmful to man. The fundamental cause of Wilson's disease is Cu toxicity. For drinking water, it might be much better if Cu would have been absent though the allowed concentration is one ppm [3].

ZINC: This element is most commonly found in metalloenzymes. Accordingly the metabolic functions of this element are more than those of any other trace elements [6]. It has many functions such as formulation of growth hormones, promotion of protein synthesis and in seed and grain maturation and production [7]. In rice plant, it has many functions such as activation of many enzymatic

reaction, close involvement in nitrogen metabolism and probable connection with the production of auxin [2]. Though Zn is a very important trace element but it has its own negative effect for human health. The most common form of Zn poisoning in humans is a nonfatal metal-fume fever caused by inhalation of Zn oxides fumes. Ingestion of acidic foods prepared in Zn-galvanised containers also results sometimes in an illness. Dermatitis can occur upon zinc chloride contact with skin [3].

SELENIUM: Selenium is considered as an element essential to plant life and it is present as a seleno-amino similar to cysteine and Leucine [3]. It is also present in several Molybdenum and Tungsten proteins which utilise Formate as Carbon source or Nitrate as Nitrogen source [9]. When the occurence of this element is at toxic level it results the so called " Alkali disease" and losses of live stock. In animals, Se poisoning produces Pneumonia and degeneration of the liver and kidneys. It may cause cancer of the liver. However, Se appears to be a natural protective agent against heavy metal toxicity to biological systems. It is reported that in human body it occurs in all the cells and tissues in various concentrations. The growth and fertility in animals and for prevention of various diseases is related to its presence [3].

MOLYBDENUM: This element is essential for plant life. The only known metallo-enzyme containing Mo in plants is nitrate reductase while in animals it is Xanthine oxidase [3]. It is associated with symbiotic nitrogen fixation and protein synthesis [7]. The function of Mo in rice plant is related to reduction of nitrate to nitrite [2]. It is known to be useful for certain Nitrogen transformation in Micro-Organisms as well as in plants [3].

CADMIUM: Cd is both toxic to plants and animals. Since it is a cummulative poison, there is concern over the possibility of increased Cadmium uptake by

plants. The role of this element in plants is not well established. However, small amount of Cadmium are added to most soils in P fertilizers [9]. Cd is closely associated with Zn both in occurance and metabolism. In biological ligands, there is a competition between Zn and Cd which results in the important implication for health. Cadmium and Cd compounds are toxic substances by all means of administration, reducing acute or chronic symptoms varying in intensity from irritations to death. Inhalation of Cd fumes, oxides and salts produces emphysema which may be followed by bronchitis. Prolonged exposure to airborne Cd frequently causes kidney damage resulting in proteinuria. The dreadful disease known as Itai-Itai is the result of water contamination by Cd from mining waste. Cadmium poisoning resulted in gross deformities and pain as bones snapped under the weight of the body which may even lead to death [3].

The recommended dietry intakes of essential and toxic elements for Adults are listed [1] :-

RECOMMENDED DAILY DIETRY INTAKES OF ESSENTIAL AND TOXIC ELEMENTS

[The values quoted refer to adults. For infants, children and adolescents, different intake values of essential elements are recommended]

ELEMENT	QUANTITY	INTAKE VALUE PER DAY
As	maximum acceptable daily load	2 mg/Kg body-weight
Ca	recommended dietry allowance	800 mg
Cd	provisional tolerable intake	1-2 mg/Kg body-weight
Cl	estimated safe and adequate intake	1700-5100 mg
Co	recommended dietry allowance	3 mg vitamin a
Cr	estimated safe and adequate intake	50-200 mg
Cu	estimated safe and adequate intake	2-3 mg
	maximum acceptable daily load	0.05-0.5 mg/Kg body-weight (provisional)
F	estimated safe and adequate intake	1.5-4.0 mg
Fe	recommended dietry allowance	10 or 18 mg (age/sex dependent)
	maximum acceptable daily load	0.8 mg/Kg body-weight
Hg	provisional tolerable intake	0.7 mg/Kg body-weight total Hg 0.5 mg/Kg body-weight methyl Hg
I	recommended dietry allowance	150 mg
K	estimated safe and adequate intake	1875-5625 mg
Mg	recommended dietry allowance	300 mg (female); 350 mg (male)
Mn	estimated safe and adequate intake	2.5-5.0 mg
Mo	estimated safe and adequate intake	0.15-0.5 mg
Na	estimated safe and adequate intake	1100-3300 mg
P	recommended dietry allowance	800 mg
Pb	provisional tolerable intake	7.1 mg/Kg body-weight
Se	estimated safe and adequate intake	50-200mg
Sn	maximum acceptable daily load	20 mg/Kg body-weight (provisional)
Zn	recommended dietry allowance	15 mg
	maximum acceptable daily load	0.3-1.0 mg/Kg body-weight (provisional)

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APPENDIX-III

SENSITIVITY AND DETECTION LIMITS OF ELEMENTS IN AAS

ELEMENT	WAVELENGTH	SBW	FLAME	SENS	IMPACT BEAD	
	nm	nm	GASES	check (a)	Sens	D. L.
B	249.7	0.7	N-Ac	600.0	12.0	1.2
Al	309.3	0.7	N-Ac	50.0 (b)	0.6	0.04
V	318.4	0.7	N-Ac	90.0 (b)	1.3	0.06
Cr	357.9	0.7	A-Ac	4.0 (e)	0.04	0.002
Mn	279.5	0.2	A-Ac	2.5 (e)	0.03	0.001
Fe	248.3	0.2	A-Ac	5.0 (e)	0.04	0.003
Co	240.7	0.2	A-Ac	7.0 (e)	0.08	0.006
Ni	232.0	0.2	A-Ac	7.0 (e)	0.04	0.004
Cu	324.8	0.7	A-Ac	4.0 (e)	0.03	0.001
Zn	213.9	0.7	A-Ac	1.0 (e)	0.011	0.0008
Se	196.0	2.0	A-Ac	30.0 (e)	0.33	0.07
Mo	313.3	0.7	N-Ac	30.0	12.0	1.2
Cd	228.8	0.7	A-Ac	1.5 (e)	0.02	0.0005

(a) Metal concentration (mg/L) in aqueous solution which will

(b) give a reading of approximately 0.2 absorbance units.

(c) Addition of alkali salt (e.g. K, La or Cs as chloride)

(d) recommended to control ionozation.

(e) The use of impact bead will improve sensitivity by about 2x.

(f) A-Ac = Air-acetylene

(g) N-Ac = Nitrous oxide-Acetylene

APPENDIX-VI

PROMINENT LINES OF THE ELEMENTS EMITTED BY THE INDUCTIVELY COUPLED PLASMA

ELEMENTS	WAVELENGTH nm	L/B Ratio	CONC. ppm	EST'D DET LIM mg/l
B	249.773	63.0	10.0	0.0048
Al	309.271	13.0	10.0	0.023
V	310.230	47.0	10.0	0.0064
Cr	205.552	49.0	10.0	0.0061
Mn	257.610	220.0	10.0	0.0014
Fe	238.204	65.0	10.0	0.0046
Co	238.892	50.0	10.0	0.0060
Ni	221.647	29.0	10.0	0.010
Cu	324.754	56.0	10.0	0.0054
Zn	213.856	170.0	10.0	0.0018
Se	196.090	40.0	100.0	0.075
Mo	202.030	38.0	10.0	0.0079
Cd	214.438	120.0	10.0	0.0025

NOTE:-

L/B Ratio - Line to Background ratio.

EST'D DET LIM - Estimated Detection Limit.

APPENDIX-V

DISCOVERY OF TRACE ELEMENTS IN PLANT NUTRITION

Trace element	Year discovered	First reported by
Manganese	1922	J. S. McHargue
Boron	1923	K. Warington
Zinc	1926	A. L. Sommer and C. B. Lipman
Copper	1932	C. B. Lipman and G. McKinney
Molybdenum	1939	D. I. Arnon and P. R. Stout
Chlorine	1954	T. C. Broyer, A. B. Carlton, C. M. Johnson, and P. R. Stout.
Sodium	1957	P. F. Brownell and J. G. Wood

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1. **MICRONUTRIENT ELEMENTS IN ACID ALFISOLS OF MEGHALAYA UNDER RICE CULTIVATION.**
   P. Nongkynrih, Paul S. Dkhar and D. T. Khathing.
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   Paul. S. Dkhar and P. Nongkynrih
   Manuscript under preparation.

5. **DETERMINATION OF TRACE ELEMENTS IN RICE PLANT USING X-RAY FLUORESCENCE TECHNIQUE.**
   Paul. S. Dkhar and P. Nongkynrih
   Manuscript under preparation.