# HEAVY METAL CONTENTS
# OF
# LOCALLY GROWN GRAINS

**Submitted**

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## Certificate

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## Abstract

Essential/non-essential heavy trace metal and macro-nutrient contents of fifteen different grains procured from local markets/fields of Punjab and NWFP were estimated and reported . The estimated non-essential metals included Cd, Cr, Pb, Hg, Ni and As, while the macro-nutrients studied were K, Na, Ca and Mg. The micronutrients investigated were Fe, Cu, Mn and Zn. The relevant soil samples were also analysed for the above mentioned metals along with their organic matter content and pH values. The food grains investigated were: wheat, rice, maize, barley, gram (white), gram (black), bean (white), bean (red), mong, masur, mash and dal chana. The data are computed at 99% ( s) confidence level for triplicate measurements and reported with an overall reproducibility within ± 1%. The study revealed that the bean and mong are good sources of macronutrients, K, Ca, Mg and Na, while gram and mash are rich sources of Fe, Zn and Cu at the level of 44.200, 49.050 and 8.125 mg/kg, respectively. Wheat has the highest Mn content in all the food grains analysed from both the provinces. It was found that, generally, all the food grains are either free from or low in heavy toxic metals burden. However, Ni and Cr contents of gram (white) and bean (red), from NWFP, were exceptionally high, at 2.710 and 3.800 mg/kg, respectively, on dry weight basis.

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CHAPTER 1

# INTRODUCTION

## 1.1 General

There is a growing international concern about human intake of toxic trace elements such as cadmium, lead, mercury and arsenic. Intake of relatively low doses of these elements over a long period can lead to malfunction of organs and chronic toxicity[1]. Toxic trace elements are in part ingested with the edible parts of agricultural and horticultural crops or derived products.

Any element could be toxic beyond certain limits. The normal "background" concentration of these elements must be known to meet the standards concerning the daily intake from food. Information on background levels provide a guideline for evaluating the effect of soil additions, such as fertilizers and sewage sludge containing these elements as well as the effect of commercial food handling and processing steps which can result in food contamination.

## 1.2 Environmental Pollution

The distribution of elements in nature, their concentrations and their movements can be seriously affected by human activities. Environmental pollution results from

Man's introduction of different substance into the marine, atmospheric or soil environment. All plants and all animals, including Man, depend ultimately upon their environment for the supply of mineral nutrients. The relation between the environment and living beings is very complex. Industrial treatment of different materials, their transport, storage and processing, as well as other of Man's activities, can influence this very sensitive relationship[2].

In case of soil pollution, the direct effectees are the plants including crops which then on consumption by Man, make the things worse for his health. Atmospheric and aqueous pollution also make direct and indirect impacts on human health with the supply of inadequate, unsafe and toxic mineral materials.

Until recently most environmental research on trace metals was based on an assessment of the total metal concentration. It has become increasingly evident that the environmental impact of a particular metal species may be more important than the total metal concentation[3]. It has been known for several decades that trace quantities of certain elements exert a positive or negative influence on plant, animal, and human life. However, more recently, greater interest has been taken with regard to the specific

role of these elements.

## 1.3 Essential Trace Elements

In food analysis, the term 'trace element' is often used to refer to those elements (mainly metals) which are present in relatively small amounts in foods and for which limits have been officially recommended or prescribed[4]. Such limits are applied in most instances because of (i) possible toxicity of the element, and (ii) the feasibility of the limit in relation to good manufacturing practice.

It has been borne out by experimental evidence that the role of heavy metal ions in living systems follows the pattern of natural availability and abundance of the same metal occurring in nature[5,6].

An element is essential when: (i) it is consistently determined to be present in all healthy living tissues within a zoological family, (ii) deficiency symptoms are noted with depletion or removal, which disappear when the elements are returned to the tissue, (iii) the deficiency symptoms should be attributed to a distinct biochemical defect (on the molecular level)[7].

It is of interest to classify the essential metals according to their biological role.

*Sodium and Potassium.* Both metals are highly concentrated in bodily fluids and are widely distributed throughout the human body. Accordingly, they are not considered as trace elements, but rather as "macroelements". Being the most mobile cations, it is not surprising that apart from an involvement in metabolic processes these ions participate in nerve impulse conduction via the brain[8].

*Magnesium and Calcium.* These are next most mobile metal ions which are widely distributed throughout the human body and thus cannot be considered as being trace metals. Magnesium ions are found complexed to nucleic acids and are necessary for nerve impulse transmissions, for muscle contractions and metabolic functions. Calcium ions having a greater affinity for oxygen-containing ligands are less mobile than magnesium ions. This results in the presence of crystalline calcium salts, e.g., phosphate and oxalate, in the circulatory blood system. The insolubility of many calcium salts is reflected by the formation of bones and teeth in which calcium is deposited as hydroxyl-apatite, $Ca_5(PO_4)_3(OH)$[5]. Thus calcium is by far the most abundant metal in the human body.

### 1.3.1 Trace Metals

The essential transition metals including zinc are

capable of forming stable bonds to fixed positions of immobile protein molecules, where they function mainly as catalyst i.e., they induce or enhance enzymetic activity. In the highly specific metalloenzymes the metal is firmly associated with the protein and often constitutes the active centre of the living cell, catalyzing only one specific reaction or type of reaction. This explains why in some cases trace concentrations have such a powerful, directive influence on the biological functions within the human body.

*Manganese.* The second most abundant metal in nature (exceeded only by iron). The chemistry of $Mn^{2+}$ resembles that of $Mg^{2+}$, both ionic species preferring weaker donors such as phosphate and carboxylate groupings to form stable bonds. It was known that many enzymes are activated by manganese in vitro, a property notably shared with magnesium. It was found that this element is involved in glucose utilization[9].

*Iron.* The most abundant transition element, is also probably the most well known metal in biological systems (haemoglobin in blood , the oxygen-carrying protein molecule of blood, regarded as the most important iron(II) complex consisting of the globin protein with four heme units attached to it).

*Cobalt.* Relatively scarce in the earth's crust, but the

human body requires vitamin $B_{12}$, which is a cobalt(III) complex, to form haemoglobin. Having the ability to occupy low symmetry sites in enzymes, cobalt(II) is an enzyme activator.

*Copper.* Long before copper was recognised as an essential element, it was shown to exist in combination with the blood protein of snails. Today it is known that Cu(I) is found in enzymes capable of carrying oxygen as hemoglobin does, and that it is actually required in the formation of this substance.

*Zinc.* One of the most abundant of the essential elements required by the human body and approximately 100 times as abundant as copper[5]. Zinc appears to be present in all animals. As with cobalt(II), zinc has the ability to occupy low symmetry sites in enzymes, and can therefore function as an essential constituent of several of them.

*Molybdenum.* This metal is envolved in electron transfer processes as in xanthine and purine oxidations of milk. Nitrogen fixation is also coupled to a molybdenum process.

## 1.4 Health Hazards Due to Toxic Trace Metals

Essential trace metals such as zinc become toxic when

the nutritional supply becomes excessive. A metal in trace amounts (smaller than 0.01% of the mass of the organism) is essential when an organism fails to grow or complete its life cycle in the absence of that metal. However, the same trace metal is toxic when concentration levels exceed those required for correct nutritional response by factors varying between 40 to 200 fold[10].

Viewed from the standpoint of environmental pollution, metals may be classified according to three criteria (i) noncritical, (ii) toxic but very insoluble or very rare, and (iii) very toxic and relatively accessible. Such a classification has been made by Wood[11], and is listed in Table 1.1.

Table 1.1 Classification of elements according to toxicity and availability

| Noncritical | | | Toxic but very insoluble | | Very toxic and relatively accessible | | |
|---|---|---|---|---|---|---|---|
| Na | C | F | Ti | Ga | Be | As | Au |
| K | P | Li | Hf | La | Co | Se | Hg |
| Mg | Fe | Rb | Zr | Os | Ni | Te | Tl |
| Ca | S | Sr | W | Rh | Cu | Pd | Pb |
| H | Cl | Al | Nb | R | Zn | Ag | Sb |
| O | Br | Si | Ta | Ru | Sn | Cd | Bi |
| N | | | Re | Ba | | Pt | |

An over view of some toxic trace metals may now be presented.

*Arsenic:*Although arsenic has been used for its medical virtues in the form of organic arsenicals over the past years for the treatment of many ailments, yet its toxicity remained well known. Soluble inorganic arsenites and arsenates are readily absorbed by the digestive tract, and the muscle tissue. Arsenite inhibits the functioning of thiol-dependent enzymes, binds to tissue protein as keratin disulfides in hair, nails, and skin. Long term ingestion of arsenic produces intestinal, skin, liver, and nerve tissue injuries. Examples of chronic poisoning have been reported for water with arsenic concentrations of 210 $\mu g/L$ to 1000 $\mu g/L$[12].

*Chromium:* Chromium is also a toxic trace element. Generally, the human body can tolrate 100-200 times its total body content of chromium without harmful effects. Chromium(VI) compounds are approximately 100 times more toxic than Cr(III) salts. The stomach acidity leads to reduction of Cr(VI) to Cr(III) of which gastrointestinal absorption is quite finite. Chromium is known to cause lung cancer.

*Copper:* Copper is an essential metal, in a number of enzymes capable of carrying oxygen, as hemoglobin does. Excessive intake of copper, however, results in its

accumulation in the liver. Generally, copper toxicity is increased by low Mo, Zn and $SO_4^{2-}$ intake. In outher words, copper toxicity or defficiency is not merely dependent upon copper intake, but also upon dietary levels of Zn, Fe and Ca[9].

*Lead:* It resembles calcium in terms of mobilization in bones. More than 90% of the lead retained in the body is in the skeleton. Although lead is a non-essential element, it is present in all tissues and organs of the human body. The larger affinity of $Pb^{2+}$ for thiol and phosphate containing ligands prevents the biosynthesis of heme and thus affects membrane permeability of kidney, liver and brain cells. This results first in reduced functioning and later complete breakdown of these tissues due to cumulative poisoning.

*Mercury:* Mercury is a non-essential but highly toxic element for living organisms. Even at low concentrations mercury and its compounds present potential health hazards due to enrichment in the food chain. Organic mercury is more toxic than inorganic mercury compounds. Mercury has no metabolic function to perform in the human body and, therefore, may be considered potentially harmful.

Studies on mercury poisoning incidents in Iraq[13], in Minamata and Niigata, Japan and in Canada (Methylmercury

Study Group, 1980) have been useful in establishing threshold levels of mercury. The toxic effects of methylmercury in humans results in neurological disturbance. Clinically observable effects in human adults occur at blood levels of 0.2-0.5 μg/ml and body concentrations of about 0.5-0.8 μg/Kg[14]. It is also known that infants are more sensitive to methylmercury exposure than adults.

*Zinc:* It is one of the most abundant essential trace element in human body. It is a consitituent of all cells, and several enzymes depend upon it as a co-factor. Concern has arisen because of the intimate connection of zinc with cadmium in the geosphere and biosphere. In aduldt human kideny, the molar abundance of cadmium can reach or exeed three-fifths that of zinc, varying widely from area to another[15]. The metabolism may be affected by zinc intake at high levels, generally exceeding the tolerance limit, causing vomiting, nausea and pain in stomach. Smaller amounts may cause gastrointestinal disturbance[16].

*Nickel:* Nickel produces fatal symptoms of poisoning when the recommended concentration exceeds. The limit recommended by Trevethick for the nickel and its salts is 1 mg/Kg. Nickel is widely distributed in very low concentrations in tissues. Serum nickel in healthy adults

about 2.6 $\mu g/dm^3$. It has been reported that in patients with acute myocardial infaction, the mean concentration of serum nickel were significantly increased through the periods of 1-36 hours after the onset of symptoms[17]. The existance of metalloprotein in human serum which is rich in nickel and does contain other detectable trace metals has also been reported[18].Nickel tetracarbonyl is several times more toxic than carbon monoxide. The usual ailments resulting from Ni toxicity are lung blood cancers.

*Cadmium:* Cadmium is found in nature in low concentrations, generally associated with zinc. Cadmium and its compounds are toxic to human. They produce acute or chronic symptoms varying in intensity from irritation to extensive disturbance finally resulting to death . There is not known biological function for cadmium in man[15].

Cadmium is known to be toxic to all systems and functions of the body, and is absorbed without regard to any previous level. Inhalation of cadmium fumes, oxides, and salts offen produces emphysema, followed by bronchitis. Prolonged exposure to cadmium cause kideny damage and also effect the heart and liver. The health effect of cadmium, both proven and probabler are, increased blood pressure, increased incidente of arteriosclerotic disease and reduced

life expectancy[19]. Cadmium taken through lungs is about 60 times more poisonous than when taken through the digestive tract.

CHAPTER 2

# ANALYTIC METHODS

## 2.1 Background

There is an urgent demand for the reliable and rapid analysis of trace elements in biological materials. The most important are metals and metalloids that are considered as essential, toxic or possibly toxic to man, animals and plants. In conjuction with similar analytical demands from other research branches, this situation has stimulated in recent decades progressively intensified development and progress manily in the field of instrumentation. Manufacturers of analytical instruments have therefore, increasingly designed and offered highly advanced systems for trace analytical purposes.

A grreat challange for analytical chemists now is therefore to achieve meaningful, i.e., from present state of the art accurate, data on a more extended scale then before. In order to achieve this target, some change in analytical philosophy by producers and users of trace analytical data is essential.

## 2.2 Various Analytical Methosds

This section deals with analytical methods that are either mainly used or still in common use but are very promising for trace metal analysis in biological materials. The methods are briefly introduced and their instrumentation and performance discussed.

Stika and Morrison[20] listed sensitivities and detection limits for various elements determined by six different techniques (Table 2.1).

Table 2.2[21] gives a summary of some important analytical techniques and their respective detection limits and relative errors under optimal conditions.

Table 2.1 Sensitivities ($\mu$g/g) and detection limits ($\mu$g/g) of various analytical methods

| Element | AES (detection limits) | NAA (sensitivity) | MS (detection limits) | AAS (sensitivity) | Chem. (detection lim.) | XRF (detection lim.) |
|---|---|---|---|---|---|---|
| Al | 0.5 | 0.004 | 0.002 | 0.8 | 0.0005 | 17 |
| As | 10 | 0.005 | 0.0006 | 0.8 | 0.01 | -- |
| Cd | 2 | 0.005 | 0.007 | 0.01 | 0.003 | 2.5 |
| Co | 0.1 | 0.01 | 0.0005 | 0.07 | 0.003 | 0.2 |
| Cr | 1 | 0.3 | 0.0005 | 0.06 | 0.007 | 0.7 |
| Cu | 0.1 | 0.002 | 0.002 | 0.04 | 0.002 | 0.4 |
| Fe | 1 | 2 | 0.0005 | 0.06 | 0.2 | 0.3 |
| Hg | 2 | 0.003 | 0.007 | 2.2 | 0.005 | -- |
| Mn | 0.1 | 0.001 | 0.0004 | 0.02 | 0.005 | 0.2 |
| Ni | 0.2 | 0.7 | 0.002 | 0.07 | 0.004 | 0.2 |
| Pb | 0.02 | 0.5 | 0.003 | 0.1 | 0.006 | 2 |
| Sb | 2 | 0.007 | 0.002 | 0.3 | 0.004 | -- |
| Se | 100 | 0.01 | 0.002 | 2 | 0.1 | -- |
| Si | 0.5 | -- | 0.002 | 2 | 0.1 | -- |
| Sn | 0.5 | 0.03 | 0.004 | 1 | 0.06 | -- |
| Zn | 5 | 0.1 | 0.002 | 0.009 | 0.1 | 2 |

AES, Arc Emission Spectroscopy; NAA, Neutron Activation Analysis; MS, Mass Spectrometry; AAS, Atomic Absorption Spectrometry; Chem, Chemical Methods; XRF, X-ray Fluorescence Spectrometry.

Table 2.2 Detechion limits and relative error by Analytic Methods

| Method | Limits of detection (g) | Relative error[a] |
|---|---|---|
| Gravimetry | $10^{-5}$ | 0.1-1 |
| Titrimetric analysis | $10^{-7}$ | 0.2-2 |
| Spectrophotometry | $10^{-8}$ | 0.5-5 |
| Fluorimetry | $10^{-9}$ | 2-10 |
| X-ray fluorescence | $10^{-7}$ | 205 |
| Electron beam microanalysis | $10^{-14}$ | 2-10 |
| Special polarography | $10^{-10}$ | 2-10 |
| Gas chromatography | $10^{-12}$ | >2 |
| Atomic absorption spectroscopy | $10^{-12}$ | >1 |
| Emission spectroscopy | $10^{-10}$ | >5 |
| Catalytic methods | $10^{-12}$ | >10 |
| Neutron activation analysis[b] | $10^{-14a}$ | >10 |
| Solid state mass spectroscopy | $10^{-16}$ | |

a Under optimal conditions

b For a neutron flux of $10^{13}$ n/cm$^2$s

### 2.2.1 Colorimetry

Colorimetric methods along with gravimetric and volumetric methods are classical analytical procedures. Many are still in use, primarily for automated techniques usuing devices such as auto analyzer[22]. In AOAC manual[23], 10 elements given out of 14 in food are determined by colorimetric method although alternate procedures have also been reported.

Most of these classical analytical procedures are tedious, requiring separation of interfering substances as well as concentrating the element of interst. These are marked by low errors of determination but their limits of detection are less than the most instrumental procedures.

Analyst of today are more interested in the instrumental techniques which are characterised by rapid multi-element capacity and greater sensitivity than in these classical analytical methods.

### 2.2.2 Emission Spectroscopy

Analysis by emission spectroscopy is the determination of an element by means of its emission spectrum, obtained from a suitable excited source. Qualitative analysis is based on the wavelengths of the lines characterstic of the element,

and quantitative analysis on the intensity of these lines.

The three standard procedures each one using a different method of excitation, are: flame spectroscopy, arc spectroscopy, and spark spectroscopy. An AC spark emission method for plant analysis has been given in AOAC manual[24]. Though, spark method is less sensitive then arc, but is more reproducible[25].

### 2.2.3 Flame Emission photometery

Flame photometers are simple in design and operation but quite limited in application. Though excellent for the determination of potossium and sodium but poor for the determination of other elements when present in complex matrices. The excitation source can be mixtures of acetylene and air, acetylene and oxygen, acetylene and nitrous oxide or argon-hydrogen-entrained air.

Matrix characteristics can introduce problems which are difficult to control, frequently requiring separation of element(s) of interest, removal of interfering ions or employment of compensation technique[26]. For biological samples lithium as an internal standard is recomended. Multi-elemental capability is possible although there are one or two detectors in most commercial instruments present.

Busch and Morrison[27] discussed the multi-elemental capability which existed and looked promising before the introduction of plasma excitation technique.

### 2.2.4 Atomic Plasma Spectroscopy

Plasma spectroscopy has revolutionised the emission spectroscopy, providing analyst with multi-element technique with excellent sensitivity and emission stability. The most common plasma is the inductively coupled plasma (ICP) whose operating principles have been discussed by Fassel and Kniseley[28]. Internal plasma temperature ranges from 6000 to 10000 K° which minimises the effect due to self-absorption and other interferences.

Sample is introduced into plasma as water aerosol. Nebulisation is done by using either crossflow, concentric or ultrasonic nebulizers. sample introduction is either by pneumatic flow or by means of peristaltic pumping. Soltanpour and Workman[29] has described gaseous sample forms (hydrides) direct introduction into plasma for arsenic, mercury and selenium.

The prominent lines of 70 elements have been identified by Winge and others[30], as emitted in excitation source of ICP. Wolnik et al[31]., and Jones[32] have given realistic detection

limits based on real samples. For most elements, detection limits with ICP were one to several orders of magnitude better than those obtained by other techniques.

### 2.2.5 Atomic Absorption Spectroscopy

Atomic-absorption spectroscopy (AAS) is the study of absorption of radiant energy by atoms. As an analytical process, it includes the conversion of combined elements to atoms, and the absorption of radiant energy by those atoms. When radiation of a selected frequency passes through an enclosure containing free atoms which can emit that frequency when excited, resonance effects are accompanied by an absorption of incident radiation and so the intensity of the tranamitted radiation is decreased.

The application of atomic absorption to analytical chemistry began in 1940's with the determination of mercury vapour in air[33], and for some years little was published on the subject, since emission methods were used widely. The combination of AAS with flame excitation, first proposed in 1955, started a unique expansion[34]. Owing to its sensitivity, specificity, element coverage, speed, precision and the initially inexpensive instrumentation, despite the fact of being a single-element method, flame AAS quickly dominated

other established analytical techniques, as is obvious by the vast number of papers published and by the adoptation of AAS in numerous laboratories. In 1970 more than 10,000 AAS instruments were in use around the world[35,36].

In 1969, the first comercially available graphite furnaces were introduced. They promised, however, for the price of a considerably lower sample throughput and a porrer precision, detection limits several orders of magnitude lower than with flame AAS.

Both techniques, flame and flameless AAS, are widely used for analysis purpose but both have one serious drawback, i.e., incapability of multi-element assay if one passes through the instrument. Flame AAS requires relatively large volume of sample while few micro-liters of sample are enough for flameless AAS. Flameless AAS is a major analytical technique for those elements and samples with ultra-trace concentrations and/or when sample volume is limited.

### 2.2.6 Activation Analysis

Activation analysis can be done by neutron or charged particle beams. As a result, the elements of interest in a sample are converted into radioactive species through nuclear reactions, and the characterstic radiations are then

measured. Neutron activation analysis is based upon the quantitative detection of radioactive species produced in sample by neutron-induced reaction. Most of the work has been done with slow, thermal neutrons from reactors and some with fast (E = 14 MeV) neutrons from neutron generators[37].

Iskander and Morad[38] have used NAA for the estimation of 11 elements in different varieties of wheat. Nadkarni and Ehmann[39] and Nadkarni et al[40]., have described the analysis of 13 elements by neutron activation. It is interesting to note that most of the certified elements in the NBS Biological Standard Referenc Materials have been determined by neutron activation

### 2.2.7 X-ray Fluorescence and Electron Probe Spectroscopy

Both the techniques are non-destructive but the sample preparation and analytical procedures are different. In X-ray fluorescence the sample is bombarded by X-rays and $K_{\alpha}$ line of the element is measured using one of the several detectors depending on the wavelength of the emitted radiation. Peterson et al[41,42] have used XRF for the determination of 10 elements in different cultivar of wheat grain.

Rasmussen and Knenek[43] have descrived electron probe technique for soil and plant tissue samples. For electron

probe analysis, the sample is made conductive, thereby complicating the procedure for the analysis of biological samples. This technique has limitations in the form of making the sample conductive, relatively poor detection limit, significant matrix effects and limited number of element determination.

### 2.2.8 Electrochemical Methods

The electrochemical techniques are polarographic, both direct (DC) and alternating (AC) current, anodic and cathodic stripping voltaqmmetry and polarography. The polarographic techniques are suitable for dilute solutions with sensitivities $10^{-5}$ - $10^{-6}$ mol/liters and improved sensitivities with inverse polarography including additional step of concentration with lower limit of sensitivity at $10^{-9}$ mol/liters.

Many elements can be determined by anodic or cathodic stripping voltametry. Salam Muhamed Retel and Petrov, S.I.,[44] have determined Cu, Pb and Cd in bread, wheat and rye flour. Satzger et al.,[45] have described procedure for the determination of Pb and Cd in cereals by differential pulse anodic stripping voltametry (DPASV).

Matson and others[46] have given determinations of

bismuth, cadmium, copper, lead, thallium and zinc in several biological samples. Wet oxidation was used with perchloric acid for sample preparation. Determination of above elements at subnanogram level was achieved with precision less than 10%. The major drawback of multi-element incapability makes this technique slow if a large number of elements were to be determined.

## 2.3 Other Analytical Techniques

There are some other analytical techniques for biological matrices such as spack source mass spectrometer, laser microprove spectrometery, gas chromatography, high-performance liquid chromatography, fluorometry and nephelometry and the use of specific-ion electrodes, but they are not widely used.

## 2.4 Comparison of AAS with other Techniques

The comparison of atomic absorption, flame emission and neutron activation methods for trace analysis of environmental and biological samples may be summarised as follows[47,48]:

1- Atomic absorption gives better results for the elements Mg, Pb, Ni, Cd, Zn, Cu, Hg, Sr, Ca, Na, K;

2- Flame emission method gives good results for the elements Li, Na, K, Rb, Cs, Ca, Tl, Mg, Cu;

3- Neutron activation analysis gives better results for the elements Mn, Cu, V, As, Br, Al, Sb, O, Ni, Cr, I, Se, Be, and some others.

It is important to note that these comparisions are not absolute. If neutronflux used for neutron activation analysis is changed appreciably one way or the other, these statements have to be readjusted.

As practical analytic tools, each technique world seem to posses certain inherent advantages that must be weighed in deciding which technique is the most appropriate for a given study.

The atomic absorption techniques obviously does not suffer from the usual shortcommings inherently present in other techniques. Hence, it is widely used as an analytic tool for heavy trace metal analysis.

# EXPERIMENTAL

## 3.1 Sample Preparation

The preparatory steps prior to trace metal determination are manifold. Numerous workers discuss this aspect from the initial purification of reagents to the release and loss of elements from and at the surface during digestion and pre-concentration procedures[49-53].

### 3.1.1 Weight Normalization

For biological and environmental materials the concentrations are often given for fresh weight. Fresh weight, however, has the disadvantage that, owing the continuous loss of moisture, even during short-term storage in freezers, the fresh weight reported may decrease considerably depending on the storage time and temperature. In case of whole grain, it is evident that the outer parts will contain less moisture than the inner parts.

These facts require, whenever possible, weight normalization. It is obvious that terms such as wet weight and actual weight in most instances cannot be used reliably for reporting analytical data. Oven-drying procedures to

constant weight at around 100 °C doubtless are excellent for robust materials and hence essential in classical analytical chemistry, but they are far from optimal for biological materials. Therefore, drying at temperatures below 100 °C, drying in a desiccator with $P_2O_5$, sometimes at slightly elevated temperatures, or freez-drying is frequently advocated as more reliable. The recommended procedure of drying by AOAC[54] is vacuum oven drying at 100 mm(Hg) pressure and at a temperature of 70 °C.

These methods, generally, provide sufficiently accurate information for weight normalization and at least arbitrary content/weight relationship. If an appropriate and properly standardized drying procedure is applied, errors arising from this source are commonly well below the average for the determination step.

### 3.1.2. Homogenization

The reproducibility of the data obtained can depend considerably on the homogeneity of materials. Prior to digestion, the cereals are required to be ground because there is a considerable difference of concentration of trace elements in outer and inner parts of the whole grain.

For dried samples like freeze dried and/or hygroscopic,

Teflon ball milling at liquid nitrogen temperature is used as proposed by Iyengar[55]. Materials which, easily fragment, can be ground in mortar and pestle. Reducing the particle size of sample to finer nature is desirable when the weight of subsample is less than 1.0 g.

Grinding procedures are connected with segregation and contamination hazards. Hand crushing and ball milling in agate reduces the chances of contamination. In Willey mills, brass fittings must be avoided when the elements to be determined are zinc and copper. Even stainless steel fittings deposit some iron to sample.

When the sample is ready for analysis, it should be kept in air-tight container in dark at lower temperature. Steyn[56] recommended prolonged storage in sterilized bottles at -5 $^{o}$C.

### 3.1.3. Digestion Methods

An important, sometimes crucial, step in the analytical procedure is the transformation of a solid or a liquid sample into an analyte solution. This often requires a more or less complete digestion of the materials to be analysed. The most frequently applied digestion techniques are critically discussed below with regard to particular advantages and disadvantages, sample throughput and cost. In

table 3.1 the principles of the digestion techniques discussed are summarised together with their cost-benefit ratios[57].

**Table 3.1 Digestion Methods**

| Method | Features | Cost-benefit ratio |
|---|---|---|
| Dry ashing, higher temp. | Simple and cheap but experience needed, less recommendable for precision analysis at lower levels. Useful for all methods | Good |
| Dry ashing, low temp. | If properly applied extremely low blank, but time consuming. Useful for all methods | Medium |
| Combustion | Complete ashing of all materials, but sometimes not free from contamination. Useful for all methods | Medium |
| Wet ashing, open systems | Very simple and inexpensive in routine use, but sometimes elemental losses and balak problems if particualr precautions are not taken. With a few limitations useful for all methods | Good to excellent |
| Wet ashing, closed systems under pressure | Good for volatile elements, useful for most methods. If properly applied very low to extremely low blanks. Caution: blow-off possible. Organometallic compounds of As are incompletely decomposed requiring further treatment | Good |

### 3.1.3.1 Dry Ashing at Higher Temperatures

If the volatility of the element to be analysed and of its compounds upto at least 823 $^{o}$K (550 $^{o}$C) is negligible, dry ashing in various, and sometimes very sophisticated types of muffle furnaces, e.g., with temperature programming[58,59] and quartz walls[60], can be the method of choice. Further, oven ashing is inexpensive and comparatively easy under mentioned conditions. If amounts at lower micrograms or even nanogram per gram (ppb) level are to be analysed, however, dry ashing becomes difficult.

Thus, if highly accurate data at the trace and ultratrace level are needed, oven ashing is usually less recommendable and may introduce errors due to contamination from the oven material (walls), dust or ashing acids such as $H_2SO_4$ or $HNO_3$. Also, substantial and irreproducible losses can occur because of adsorption on the walls of the crucibles used.

### 3.1.3.2 Dry Ashing at lower Temperatures

From the point of view of contamination from the laboratory environment, trace element losses, adsorption and blank minimization, the so-called lowtemperature ashing (LTA) in microwave-excited oxygen plasmas, with partial pressures

≤ 1 Torr, at average temperatures below 423 $^{o}$K (150 $^{o}$C)[61], is often said to be the most promising approach for trace and ultratrace analysis.

Limitations of this method are the expensive ashing devices and the usually lengthy procedure. As the excited plasma only reacts at the sample surface, the latter can pose problems in preparing samples for low-temperature ashing. If materials such as bones and plants contain large amounts of inorganic constituents, a complete ashing is extremely time consuming[62].

### 3.1.3.3 Combustion

Another technique is the combustion of organic material in a pure oxygen stream, which is reported to be especially effective for fatty materials, for example adipose tissue.

Burning of a solid material in a quartz dish, a solution or a slurry by direct introduction into an oxygen-hydrogen flame is known as Wickbold combustion. This method can be used successfully prior to the determination of mercury in biological materials[63,64]. A slightly modified Wickbold system was also shown to be useful prior to the analysis of other elements[65].

A limitation of combustion methods is comparatively low sample throughput. Methods of this kind, however, are useful within the concept of the application of independent analytical principles, and also of different alternative ashing procedures, to confirm independently trace analytical results[66-70].

### 3.1.3.4 Wet Ashing (Open System)

Wet digestion of biological materials, frequently with a single acid, mainly $HNO_3$, with the aid of UV irradiation[72], and also with acid mixtures such as $HNO_3$-$HClO_4$, $HNO_3$-$HClO_4$-$H_2SO_4$, $H_2SO_4$-$H_2O_2$ and $H_2O_2$-$HNO_3$ has been extensively treated in the literature[71-73].

Wet digestion is still the most frequently used digestion technique prior to all trace analytical determination methods. The reason is the simplicity and adaptability of wet digestion procedures to nearly every analytical task. Also, a high sample throughput can be achieved. Digestion devices consist of simple vessels of various forms or tubes made from laboratory glass, pyrex or quartz and of various reflux systems to enhance the digestion potential and to minimize as far as possible reagent consumption and trace element losses and contamination[74,75].

Disadvantages are the high consumption of acids in conjugation with elevated temperatures ( >300 $^{o}$C) if a complete digestion is required and related contamination problems from the walls of the vessels and the reagents.

### 3.1.3.5 Wet Ashing (Closed Systems Under Pressure)

In ultratrace analysis and particularly the analysis of volatile elements, pressurized decomposition was found to be advantageous in comparison with most open digestion procedures[76-78]. Commonly $HNO_3$ is used as the main oxident, but addition of other acids for particular purposes (HF, $HClO_4$, $H_2SO_4$) have also been reported[68,76]. As the oxidation potential of $HNO_3$ increases significantly at elevated temperature and pressure, the consumption of acid, preferably from sub-boiling distillation, is usually lower than for open wet digestion. This is also advantageous if blanks have to be extremely low.

In case of incomplete digestion other treatments are required, e.g., prolonged digestion time, at elevated temperature with the addition of other reagents[79]. These additional treatments can increase blanks, clearly demonstrating the limitations of pre-treatment procedures, particularly of pressurized decomposition, in trace and

ultratrace analysis. In this context it is emphasized that the after-treatment by UV irradiation adds no additional sources of contamination[80].

## 3.2 EXPERIMENTAL PROCEDURE

For the purpose of weight normalization, the cereal samples were dried in electric oven maintained at 40 ± 1 °C. Normally, the procedure required 20-24 h. during which a constant weight of the dried sample was obtained.

After complete removal of moisture, the sample was ground in a mortar until fine powder was obtained. An exactly weighed amount of 1.00 g. of each sample was transferred to a china dish (100 ml.) and 10.0 ml. of 65% nitric acid along with 2.5 ml. of 60% perchloric acid was added to it. The dish was subsequently placed for about 30 min. in the oven maintained at 40 ± 1 °C. The digested sample was diluted with distilled water and transferred, with constant and careful washings, to a measuring flask (50.0 ml.) by suitably making up the volume. This solution was aspirated directly onto the atomic absorption spectrophotometer for the estimation of trace metal content.

Water extract of the soil samples was used for the estimatioin of trace metals. An exactly weighed (10.0 g.)

sample of soil already ground and passed through 0.2 mm sieve was taken into a 200 ml. erlenmeyer flask and 40.0 ml of distilled water was added to it. Flask was shaken for about 10 minutes then left to stand for about one hour to settle down the particles. The extract was filtered out and the volume was made upto 50 ml. This extract was directly aspirated onto the atomic absorption spectrophotometer.

### 3.2.1 Organic Matter Determination

For the determination of organic matter in the soil the method employed was 'Rapid Wet Oxidation Method'. This method was similar to the wet combustion methods for quantitative carbon determination but less drastic techniques were employed so that only a partial oxidation resulted. Chromic acid (from potassium dichromate and sulphuric acid) was the usual oxidizing agent. Recovery compared with dry combustion methods ranged from 60-90%, it was commonly 75-80% for surface soils. Due to the range in recovery values it was preferable to quote the figures as such rather than use a conversion factor. High concentrations of chloride will interfere by giving organic carbon values higher than actual amount[81].

An exactly weighed (1.0g.) sample of soil already ground

and passed through 0.2 mm. sieve was taken in a 500 ml. erlenmeyer flask. Then, 10.0 ml. of 1 N potassium dichromate was added to this followed by the addition of 20.0 ml. of concentrated sulphuric acid. It was shaken gently for 1 minute and then left to stand as such on a sheet of asbestos for about 30 minutes to cool (soil digest A).

In the mean time, the ferrous sulphate sulphate solution was standardized by titrating it against a known volume of 1 N potassium dichromate as described bellow:

For the standardization of ferrous sulphate solution, 10.0 ml. of potassium dichromate was taken in a 500 ml. Erlenmeyer flask, 20.0 ml. of concentrated sulphuric acid, 200.0 ml. of distilled water, 10.0 ml. of concentrated phosphoric acid and 15-20 drops of 1% diphenylamine indicator (made up in concentrated sulphuric acid) were added to the flask. Ferrous sulphate solution from a 50 ml. burrette was titrated— the purple colour intensified and flushed to sharp green, indicating the end point.

The soil digest A was examined after 30 minutes, the colour of the solution was orange ( in case of green colour, the use of half the quantity of soil was recommended). Then, 200.0 ml distilled water, 10.0 ml. phosphoric acid and 15-20 drops of diphenylamine indicator solution were added to soil

digest A. The excess dichromate was titrated by the addition of standardized sulphate solution. in the beginning, blue colour changed to purple and it intensified with the addition of ferrous sulphate. At this point, ferrous sulphate was added dropwise until the purple colour flushed to green. The results were corrected by subtracting the readings of the blank run.

### 3.3 Adjustment of Analytical Conditions

All absorption measurements were made with a Shimadzu Atomic Absorption Spectrophotometer, model AA-670 and Hitachi Atomic Absorption Spectrophotometer, model 170-10. The standard analytical conditions established for the quantification of various trace metals are listed in table 3.2.

**Table 3.2 Standard Analytical Conditions Established for AAS Analysis**

| Code No. | Elements | Wave-length (nm) | HC lamp Current (mA) | Slit Width (nm) | Type of flame | Fuel flo rate (L/min) |
|---|---|---|---|---|---|---|
| 1. | As | 193.7 | 6 | 0.6 | Ar-$H_2$ | 4.2 |
| 2. | Ca | 442.7 | 6 | 0.5 | Air-$C_2H_2$ | 2.0 |
| 3. | Cd | 228.8 | 4 | 0.3 | " | 1.8 |
| 4. | Cr | 357.9 | 5 | 0.5 | " | 2.6 |
| 5. | Cu | 324.8 | 3 | 0.5 | " | 1.8 |
| 6. | Fe | 248.3 | 8 | 0.2 | " | 2.0 |
| 7. | Hg | 253.7 | 2 | 0.7 | Ar-$H_2$ | 2.0 |
| 8. | K | 766.5 | 5 | 0.5 | Air-$C_2H_2$ | 1.9 |
| 9. | Mg | 285.2 | 4 | 0.5 | " | 1.6 |
| 10. | Mn | 279.5 | 5 | 0.4 | " | 1.9 |
| 11. | Na | 589.0 | 6 | 0.5 | " | 1.6 |
| 12. | Ni | 232.0 | 4 | 0.15 | " | 1.7 |
| 13. | Pb | 217.0 | 7 | 0.3 | " | 1.8 |
| 14. | Zn | 213.9 | 4 | 0.5 | " | 2.0 |

### 3.3.1 Instrumental Specifications

The Shimadzu AAS is a high speed, dual frequency, simultaneous photometric system with automatic background correction. Other salient features of the equipment are automatic selection of optimum operational conditions, automatic recording of the data, including the calibration curve and top level data-processing function to ensure high precision and high accuracy. Lamp position, detector gain and beam balance are all adjusted automatically with flexibility to exclude deviant data in repeated analyses. The output is flexible in terms of holding of transient signals and their memory storage.

CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Results

Table 4.2 - 4.11

**Table 4.1 Description of samples from Punjab and NWFP with codes**

| Sample Code (Punjab) | Sample Code (NWFP) | Sample Description |
|---|---|---|
| 01 P | 01 N | Wheat/*Tritcum aestivum* |
| 02 P | 02 N | Rice/*Oryza sativa* |
| 03 P | 03 N | Maize/*Zea mays* |
| 04 P | 04 N | Barley/*Hordeum vulgare* |
| 05 P | 05 N | Gram white/*Cicer arietinum* (sead white) |
| 06 P | 06 N | Gram black/*Cicer arietinum* (sead black) |
| 07 P | 07 N | Bean red/*Phaeolus vulgaris* |
| 08 P | 08 N | Bean white/*Phaseolus lunatus* |
| 09 P | 09 N | Mong washed/*Vigna radiata* |
| 10 P | 10 N | Mong whole/*Vigna radiata* |
| 11 P | 11 N | Masur washed/*Lens culinaris* |
| 12 P | 12 N | Masur whole/*Lens culinaris* |
| 13 P | 13 N | Mash washed/*Vinga nungo* |
| 14 P | 14 N | Mash whole/*Vinga nungo* |
| 15 P | 15 N | Dal chana/*Cicer arietinum* |

Table 4.2 Concentration of Macronutrients (mg %, dry weight) for various Grains from Punjab

| SAMPLE CODE | LEVEL | K | Na | Ca | Mg |
|---|---|---|---|---|---|
| 01 P | $\bar{x} \pm s$ | 5.215 ±0.048 | 0.062 ±0.001 | 0.876 ±0.007 | 1.850 ±0.021 |
| 02 P | $\bar{x} \pm s$ | 1.750 ±0.011 | 0.030 ±0.001 | 0.089 ±0.001 | 0.357 ±0.003 |
| 03 P | $\bar{x} \pm s$ | 3.875 ±0.028 | 0.079 ±0.001 | 0.141 ±0.002 | 2.125 ±0.018 |
| 04 P | $\bar{x} \pm s$ | 6.875 ±0.057 | 0.408 ±0.004 | 0.465 ±0.003 | 2.875 ±0.019 |
| 05 P | $\bar{x} \pm s$ | 10.125 ±0.100 | 0.063 ±0.001 | 0.520 ±0.005 | 2.950 ±0.019 |
| 06 P | $\bar{x} \pm s$ | 13.250 ±0.112 | 0.052 ±0.001 | 0.661 ±0.005 | 2.925 ±0.021 |
| 07 P | $\bar{x} \pm s$ | 13.125 ±0.117 | 0.097 ±0.001 | 0.487 ±0.004 | 3.850 ±0.028 |
| 08 P | $\bar{x} \pm s$ | 14.625 ±0.128 | 0.317 ±0.001 | 0.527 ±0.005 | 3.425 ±0.009 |
| 09 P | $\bar{x} \pm s$ | 14.250 ±0.127 | 0.017 ±0.001 | 0.320 ±0.003 | 2.425 ±0.021 |
| 10 P | $\bar{x} \pm s$ | 14.500 ±0.129 | 0.031 ±0.001 | 0.532 ±0.005 | 3.650 ±0.027 |
| 11 P | $\bar{x} \pm s$ | 11.250 ±0.110 | 0.045 ±0.001 | 0.311 ±0.003 | 1.950 ±0.011 |
| 12 P | $\bar{x} \pm s$ | 9.125 ±0.087 | 0.053 ±0.001 | 0.446 ±0.004 | 2.500 ±0.021 |
| 13 P | $\bar{x} \pm s$ | 10.375 ±0.100 | 0.151 ±0.002 | 0.426 ±0.004 | 4.250 ±0.038 |
| 14 P | $\bar{x} \pm s$ | 12.750 ±0.113 | 0.257 ±0.002 | 0.478 ±0.006 | 4.500 ±0.039 |
| 15 P | $\bar{x} \pm s$ | 11.250 ±0.103 | 0.102 ±0.001 | 0.423 ±0.004 | 2.625 ±0.019 |

Table 4.3 Concentration of Micronutrients (mg/kg, dry weight) for various Grains from Punjab

| SAMPLE CODE | LEVEL | Zn | Cu | Mn | Fe |
|---|---|---|---|---|---|
| 01 P | $\bar{x} \pm s$ | 30.950±0.287 | 2.600±0.020 | 31.500±0.300 | 16.305±0.152 |
| 02 P | $\bar{x} \pm s$ | 17.500±0.157 | 0.819±0.007 | 8.950±0.079 | 4.400±0.038 |
| 03 P | $\bar{x} \pm s$ | 23.580±0.219 | 1.750±0.010 | 7.850±0.071 | 13.051±0.111 |
| 04 P | $\bar{x} \pm s$ | 29.051±0.199 | 3.259±0.031 | 22.450±0.200 | 22.350±0.189 |
| 05 P | $\bar{x} \pm s$ | 37.050±0.313 | 4.250±0.048 | 29.350±0.217 | 23.450±0.187 |
| 06 P | $\bar{x} \pm s$ | 35.500±0.215 | 5.725±0.050 | 24.505±0.213 | 34.750±0.279 |
| 07 P | $\bar{x} \pm s$ | 37.450±0.312 | 6.720±0.062 | 13.500±0.111 | 21.750±0.199 |
| 08 P | $\bar{x} \pm s$ | 32.400±0.270 | 4.350±0.043 | 16.850±0.153 | 31.950±0.287 |
| 09 P | $\bar{x} \pm s$ | 24.550±0.200 | 8.250±0.072 | 11.750±0.100 | 15.625±0.143 |
| 10 P | $\bar{x} \pm s$ | 33.650±0.310 | 8.150±0.073 | 16.750±0.113 | 22.903±0.200 |
| 11 P | $\bar{x} \pm s$ | 25.375±0.213 | 7.500±0.068 | 17.250±0.129 | 27.051±0.201 |
| 12 P | $\bar{x} \pm s$ | 32.426±0.288 | 8.250±0.069 | 13.244±0.112 | 37.150±0.288 |
| 13 P | $\bar{x} \pm s$ | 23.750±0.289 | 5.815±0.052 | 16.654±0.150 | 40.250±0.370 |
| 14 P | $\bar{x} \pm s$ | 19.723±0.111 | 7.250±0.070 | 20.352±0.200 | 42.251±0.372 |
| 15 P | $\bar{x} \pm s$ | 14.750±0.121 | 6.350±0.061 | 17.755±0.161 | 34.350±0.301 |

Table 4.4 Concentration of Toxic Metals (mg/kg, dry weight) for various Grains from Punjab

| SAMPLE CODE | LEVEL | Cd | Cr | Pb | Hg | Ni | As |
|---|---|---|---|---|---|---|---|
| 01 P | $\bar{x}$ | 0.070 | 1.300 | 0.450 | 0.100 | 0.055 | 0.120 |
| | $\pm s$ | ±0.001 | ±0.010 | ±0.004 | ±0.001 | ±0.001 | ±0.001 |
| 02 P | $\bar{x}$ | 0.300 | 1.150 | 0.500 | 0.003 | 1.115 | 0.055 |
| | $\pm s$ | ±0.002 | ±0.011 | ±0.002 | ±0.001 | ±0.011 | ±0.001 |
| 03 P | $\bar{x}$ | 0.305 | * | 1.400 | 0.125 | 1.870 | 0.115 |
| | $\pm s$ | ±0.003 | | ±0.010 | ±0.001 | ±0.020 | ±0.001 |
| 04 P | $\bar{x}$ | 0.250 | 1.250 | 0.200 | 0.020 | 1.450 | 0.105 |
| | $\pm s$ | ±0.002 | ±0.010 | ±0.002 | ±0.001 | ±0.013 | ±0.001 |
| 05 P | $\bar{x}$ | 0.250 | 0.070 | 0.250 | 0.007 | 1.490 | * |
| | $\pm s$ | ±0.002 | ±0.001 | ±0.002 | ±0.000 | ±0.011 | |
| 06 P | $\bar{x}$ | 0.050 | 0.350 | 1.105 | 0.105 | 1.300 | 0.025 |
| | $\pm s$ | ±0.001 | ±0.002 | ±0.010 | ±0.001 | ±0.013 | ±0.001 |
| 07 P | $\bar{x}$ | 0.200 | 0.450 | 0.325 | 0.055 | 0.350 | 0.005 |
| | $\pm s$ | ±0.002 | ±0.003 | ±0.003 | ±0.001 | ±0.003 | ±0.001 |
| 08 P | $\bar{x}$ | 0.150 | 0.450 | 1.250 | 0.960 | 2.050 | 0.010 |
| | $\pm s$ | ±0.001 | ±0.003 | ±0.012 | ±0.008 | ±0.020 | ±0.001 |
| 09 P | $\bar{x}$ | 0.200 | 0.600 | 1.150 | 0.020 | 0.178 | * |
| | $\pm s$ | ±0.002 | ±0.005 | ±0.015 | ±0.001 | ±0.002 | |
| 10 P | $\bar{x}$ | 0.215 | 0.100 | 1.400 | * | 0.193 | 0.100 |
| | $\pm s$ | ±0.002 | ±0.001 | ±0.012 | | ±0.002 | ±0.001 |
| 11 P | $\bar{x}$ | * | 0.040 | 1.105 | 0.010 | 2.450 | * |
| | $\pm s$ | | ±0.001 | ±0.010 | ±0.000 | ±0.022 | |
| 12 P | $\bar{x}$ | * | 0.640 | 0.100 | 0.046 | 1.100 | 0.021 |
| | $\pm s$ | | ±0.005 | ±0.001 | ±0.001 | ±0.012 | ±0.001 |
| 13 P | $\bar{x}$ | 0.100 | * | 1.900 | 0.050 | 1.160 | 0.011 |
| | $\pm s$ | ±0.001 | | ±0.009 | ±0.001 | ±0.012 | ±0.001 |
| 14 P | $\bar{x}$ | 0.050 | 0.250 | 1.950 | 0.052 | 1.108 | * |
| | $\pm s$ | ±0.001 | ±0.002 | ±0.009 | ±0.001 | ±0.012 | |
| 15 P | $\bar{x}$ | 0.051 | 0.650 | 0.050 | 0.071 | 2.178 | 0.005 |
| | $\pm s$ | ±0.001 | ±0.005 | ±0.001 | ±0.001 | ±0.020 | ±0.000 |

* Below detection limit.

**Table 4.5 Concentration of Macronutrients (mg %, dry weight) for various Grains from NWFP**

| SAMPLE CODE | LEVEL | K | Na | Ca | Mg |
|---|---|---|---|---|---|
| 01 N | $\bar{x} \pm s$ | 4.562±0.041 | 0.036±0.001 | 1.675±0.011 | 1.875±0.009 |
| 02 N | $\bar{x} \pm s$ | 1.695±0.010 | 0.039±0.001 | 0.222±0.002 | 0.504±0.008 |
| 03 N | $\bar{x} \pm s$ | 3.500±0.028 | 0.096±0.001 | 0.176±0.001 | 1.750±0.011 |
| 04 N | $\bar{x} \pm s$ | 5.562±0.049 | 0.419±0.004 | 0.553±0.004 | 2.178±0.020 |
| 05 N | $\bar{x} \pm s$ | 9.750±0.081 | 0.046±0.001 | 1.155±0.011 | 2.625±0.021 |
| 06 N | $\bar{x} \pm s$ | 10.300±0.093 | 0.066±0.001 | 1.575±0.014 | 2.635±0.023 |
| 07 N | $\bar{x} \pm s$ | 14.750±0.098 | 0.034±0.001 | 1.441±0.011 | 3.150±0.030 |
| 08 N | $\bar{x} \pm s$ | 14.875±0.097 | 0.486±0.004 | 0.938±0.008 | 3.750±0.031 |
| 09 N | $\bar{x} \pm s$ | 15.000±0.099 | 0.028±0.001 | 0.503±0.004 | 3.125±0.030 |
| 10 N | $\bar{x} \pm s$ | 13.000±0.110 | 0.032±0.001 | 0.804±0.007 | 3.750±0.032 |
| 11 N | $\bar{x} \pm s$ | 9.500±0.008 | 0.023±0.001 | 0.619±0.005 | 2.075±0.019 |
| 12 N | $\bar{x} \pm s$ | 8.750±0.008 | 0.041±0.001 | 0.921±0.009 | 2.126±0.018 |
| 13 N | $\bar{x} \pm s$ | 13.520±0.091 | 0.111±0.002 | 0.637±0.006 | 4.100±0.031 |
| 14 N | $\bar{x} \pm s$ | 12.250±0.101 | 0.215±0.002 | 0.653±0.006 | 4.250±0.032 |
| 15 N | $\bar{x} \pm s$ | 10.125±0.100 | 0.075±0.001 | 1.005±0.012 | 2.735±0.021 |

**Table 4.6 Concentration of Micronutrients (mg/kg,dry weight) for various Grains from NWFP**

| SAMPLE CODE | LEVEL | Zn | Cu | Mn | Fe |
|---|---|---|---|---|---|
| 01 N | $\bar{x}.\pm$ s | 25.030±0.280 | 4.061±0.038 | 32.675±0.011 | 25.025±0.253 |
| 02 N | $\bar{x}$ ± s | 7.501±0.071 | 3.100±0.031 | 12.975±0.111 | 12.400±0.111 |
| 03 N | $\bar{x}$ ± s | 11.255±0.101 | 3.250±0.034 | 10.850±0.098 | 32.257±0.319 |
| 04 N | $\bar{x}$ ± s | 13.750±0.125 | 3.755±0.035 | 21.475±0.021 | 15.950±0.148 |
| 05 N | $\bar{x}$ ± s | 25.050±0.300 | 5.000±0.051 | 27.200±0.265 | 31.900±0.300 |
| 06 N | $\bar{x}$ ± s | 49.050±0.487 | 5.160±0.048 | 28.325±0.263 | 44.200±0.310 |
| 07 N | $\bar{x}$ ± s | 36.250±0.313 | 5.625±0.049 | 16.675±0.161 | 43.600±0.299 |
| 08 N | $\bar{x}$ ± s | 27.500±0.270 | 6.250±0.047 | 17.725±0.153 | 36.875±0.312 |
| 09 N | $\bar{x}$ ± s | 20.200±0.198 | 7.505±0.070 | 16.525±0.150 | 30.275±0.290 |
| 10 N | $\bar{x}$ ± s | 21.250±0.261 | 7.500±0.069 | 16.100±0.152 | 29.453±0.281 |
| 11 N | $\bar{x}$ ± s | 20.500±0.198 | 6.875±0.065 | 16.100±0.125 | 29.453±0.218 |
| 12 N | $\bar{x}$ ± s | 27.250±0.263 | 7.521±0.063 | 21.352±0.213 | 36.972±0.312 |
| 13 N | $\bar{x}$ ± s | 13.725±0.124 | 8.125±0.081 | 13.651±0.123 | 42.853±0.299 |
| 14 N | $\bar{x}$ ± s | 12.500±0.111 | 5.620±0.049 | 18.452±0.151 | 35.361±0.302 |
| 15 N | $\bar{x}$ ± s | 10.000±0.098 | 5.625±0.049 | 19.828±0.199 | 32.500±0.319 |

Table 4.7 Concentration of Toxic Metals (mg/kg, dry weight) for varios Grains from NWFP

| SAMPLE CODE | LEVEL | Cd | Cr | Pb | Hg | Ni | As |
|---|---|---|---|---|---|---|---|
| 01 N | $\bar{x}$ | 0.050 | 1.250 | 2.195 | 0.027 | 0.750 | 0.250 |
| | $\pm s$ | ±0.001 | ±0.010 | ±0.019 | ±0.001 | ±0.006 | ±0.002 |
| 02 N | $\bar{x}$ | 0.400 | 0.450 | 0.350 | 0.056 | 1.615 | 0.167 |
| | $\pm s$ | ±0.003 | ±0.004 | ±0.003 | ±0.001 | ±0.015 | ±0.002 |
| 03 N | $\bar{x}$ | 0.475 | 1.350 | 1.325 | 0.043 | 1.870 | * |
| | $\pm s$ | ±0.003 | ±0.012 | ±0.011 | ±0.001 | ±0.015 | |
| 04 N | $\bar{x}$ | 0.150 | 1.700 | 1.650 | * | 2.225 | * |
| | $\pm s$ | ±0.001 | ±0.015 | ±0.012 | | ±0.020 | |
| 05 N | $\bar{x}$ | 0.425 | 2.775 | 1.850 | 0.046 | 2.710 | 0.032 |
| | $\pm s$ | ±0.002 | ±0.025 | ±0.013 | ±0.001 | ±0.021 | ±0.001 |
| 06 N | $\bar{x}$ | 0.225 | 2.200 | 2.250 | 0.361 | 2.100 | 0.025 |
| | $\pm s$ | ±0.001 | ±0.02 | ±0.020 | ±0.003 | ±0.098 | ±0.001 |
| 07 N | $\bar{x}$ | 0.275 | 3.800 | 2.167 | 1.003 | 2.575 | * |
| | $\pm s$ | ±0.002 | ±0.035 | ±0.021 | ±0.010 | ±0.098 | |
| 08 N | $\bar{x}$ | 0.257 | 2.700 | 2.117 | 1.213 | 2.575 | 0.010 |
| | $\pm s$ | ±0.002 | ±0.026 | ±0.020 | ±0.010 | ±0.021 | ±0.000 |
| 09 N | $\bar{x}$ | 0.075 | 1.950 | 2.375 | * | 1.301 | 0.036 |
| | $\pm s$ | ±0.001 | ±0.018 | ±0.021 | | ±0.010 | ±0.001 |
| 10 N | $\bar{x}$ | 0.125 | 1.025 | 2.925 | * | 2.150 | 0.235 |
| | $\pm s$ | ±0.001 | ±0.010 | ±0.025 | | ±0.020 | ±0.002 |
| 11 N | $\bar{x}$ | * | 2.425 | 2.775 | 0.025 | 2.450 | * |
| | $\pm s$ | | ±0.024 | ±0.025 | ±0.001 | ±0.020 | |
| 12 N | $\bar{x}$ | 0.100 | 2.125 | 2.925 | 0.027 | 2.362 | 0.021 |
| | $\pm s$ | ±0.001 | ±0.021 | ±0.025 | ±0.001 | ±0.021 | ±0.001 |
| 13 N | $\bar{x}$ | 0.050 | 1.200 | 1.732 | * | 0.650 | * |
| | $\pm s$ | ±0.001 | ±0.009 | ±0.013 | | ±0.005 | |
| 14 N | $\bar{x}$ | * | 2.315 | 2.091 | 0.013 | 1.133 | * |
| | $\pm s$ | | ±0.020 | ±0.020 | ±0.000 | ±0.010 | |
| 15 N | $\bar{x}$ | 0.050 | 0.650 | 2.951 | 0.036 | 2.510 | * |
| | $\pm s$ | ±0.001 | ±0.005 | ±0.019 | ±0.001 | ±0.020 | |

* Below detection limit.

Table 4.8 Concentration of Macronutrients (mg/kg, dry weigt) for various Soil Samples

| SAMPLE CODE | LEVEL | Na | K | Ca | Mg |
|---|---|---|---|---|---|
| 01 SP* | $\bar{x} \pm s$ | 122.50 ±1.21 | 75.50 ±0.68 | 150.85 ±1.42 | 175.00 ±1.69 |
| 02 SP | $\bar{x} \pm s$ | 135.75 ±1.28 | 89.50 ±0.79 | 178.50 ±0.63 | 165.00 ±0.59 |
| 03 SP | $\bar{x} \pm s$ | 189.50 ±1.76 | 46.50 ±0.41 | 168.05 ±0.39 | 140.50 ±1.40 |
| 04 SP | $\bar{x} \pm s$ | 96.50 ±0.95 | 58.75 ±0.39 | 123.50 ±1.18 | 110.50 ±1.11 |
| 05 SN** | $\bar{x} \pm s$ | 137.50 ±1.20 | 101.50 ±0.92 | 160.50 ±0.52 | 73.50 ±0.69 |
| 06 SN | $\bar{x} \pm s$ | 89.35 ±0.79 | 65.50 ±0.60 | 185.50 ±1.62 | 63.50 ±0.58 |
| 07 SN | $\bar{x} \pm s$ | 103.50 ±1.01 | 70.50 ±0.69 | 110.50 ±1.00 | 180.50 ±1.35 |

* Samples from Punjab.
** Samples from NWFP.

Table 4.9 Concentration of Micronutrients (mg/kg, dry weight) for various Soil Samples

| SAMPLE CODE | LEVEL | Zn | Cu | Mn | Fe |
|---|---|---|---|---|---|
| 01 SP** | $\bar{x} \pm s$ | 0.100 ±0.001 | 0.130 ±0.001 | * | 0.285 ±0.002 |
| 02 SP | $\bar{x} \pm s$ | 0.200 ±0.002 | 0.500 ±0.001 | 0.010 ±0.000 | 0.050 ±0.001 |
| 03 SP | $\bar{x} \pm s$ | 0.050 ±0.001 | 0.005 ±0.001 | * | 0.010 ±0.001 |
| 04 SP | $\bar{x} \pm s$ | 0.020 ±0.001 | 0.010 ±0.000 | 0.001 ±0.000 | 0.020 ±0.001 |
| 05 SN*** | $\bar{x} \pm s$ | 0.300 ±0.001 | 0.002 ±0.000 | 0.001 ±0.000 | 0.010 ±0.000 |
| 06 SN | $\bar{x} \pm s$ | * | 0.004 ±0.000 | * | 0.002 ±0.000 |
| 07 SN | $\bar{x} \pm s$ | 0.020 ±0.001 | 0.010 ±0.000 | ** | 0.500 ±0.010 |

* Below detection limit.

** Samples from Punjab.

*** Samples from NWFP.

**Table 4.10 Concentration of Toxic Metals (mg/kg, dry weight) for various Soil Samples**

| SAMPLE CODE | LEVEL | Cd | Cr | Pb | Hg | Ni | As |
|---|---|---|---|---|---|---|---|
| 01 SP** | $\bar{x}$ | * | * | * | 0.050 | 1.050 | * |
| | ±s | | | | 0.001 | 0.009 | |
| 02 SP | $\bar{x}$ | * | * | 0.001 | * | * | * |
| | ±s | | | 0.000 | | | |
| 03 SP | $\bar{x}$ | * | * | * | * | 0.010 | * |
| | ±s | | | | | ±0.001 | |
| 04 SP | $\bar{x}$ | * | 0.001 | * | * | 0.002 | * |
| | ±s | | ±0.000 | | | ±0.000 | |
| 05 SN*** | $\bar{x}$ | * | * | 0.001 | * | ** | * |
| | ±s | | | ±0.000 | | | |
| 06 SN | $\bar{x}$ | * | * | * | 0.001 | 0.001 | * |
| | ±s | | | | ±0.000 | ±0.000 | |
| 07 SN | $\bar{x}$ | * | * | 0.001 | * | 0.001 | * |
| | ±s | | | ±0.000 | | ±0.000 | |

* Below detection limit.
** Samples from Punjab.
*** Samples from NWFP.

Table 4.11 Organic Matter Content and pH of various Soil Samples

| SAMPLE CODE | pH (± 0.01) | ORGANIC MATTER (%) (± s) |
|---|---|---|
| 01 SP * | 7.96 | 8.73 ±0.081 |
| 02 SP | 8.01 | 12.50 ±0.113 |
| 03 SP | 8.10 | 18.25 ±0.125 |
| 04 SP | 7.81 | 25.50 ±0.270 |
| 05 SN ** | 8.01 | 9.75 ±0.087 |
| 06 SN | 8.20 | 7.51 ±0.070 |
| 07 SN | 7.85 | 10.25 ±0.100 |

* Samples from Punjab.
** Samples from NWFP.

## 4.2 Figures

Figure 4.1 - 4.6

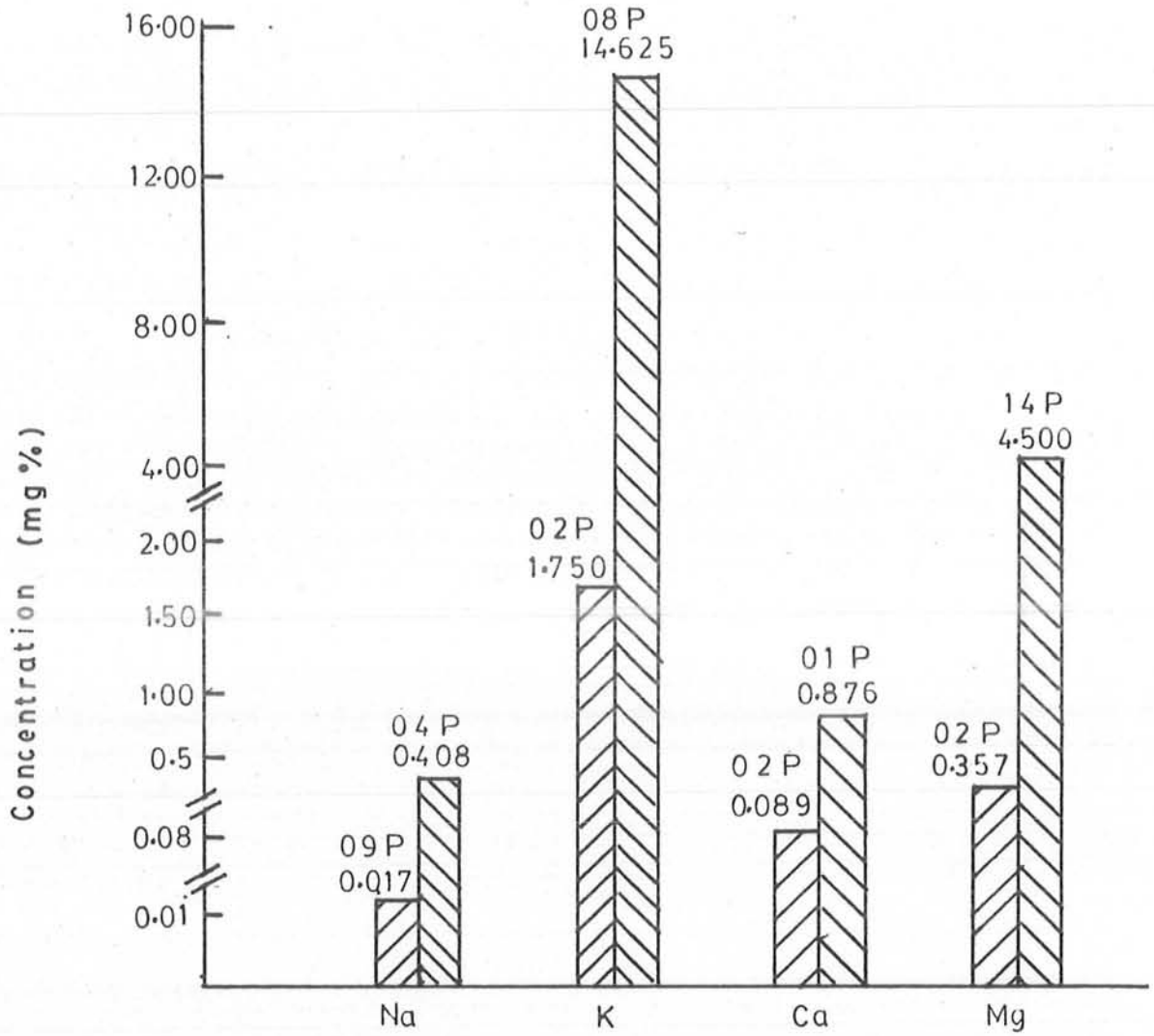



Fig 4.1 Minimum and maximum levels of macronutrients in various grains (sample code P) from Punjab

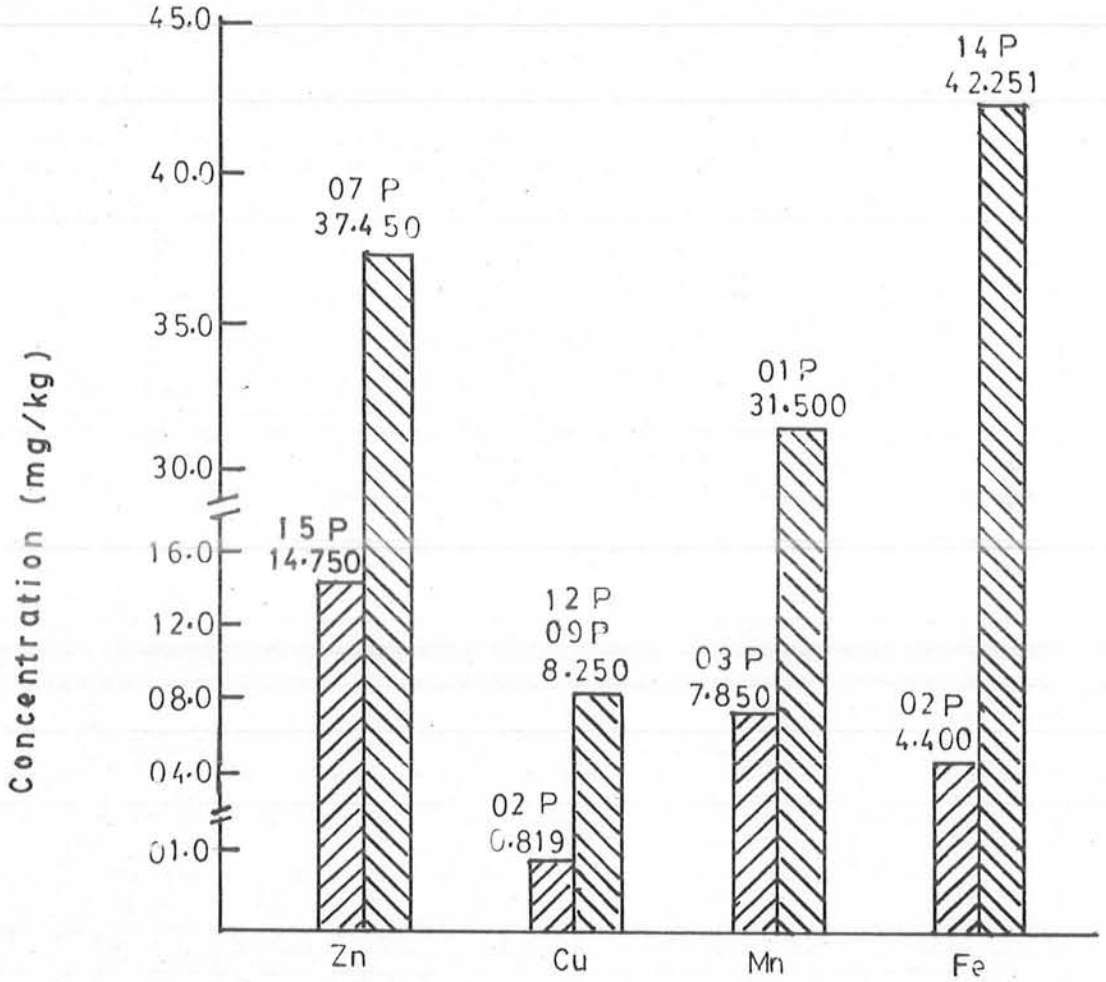
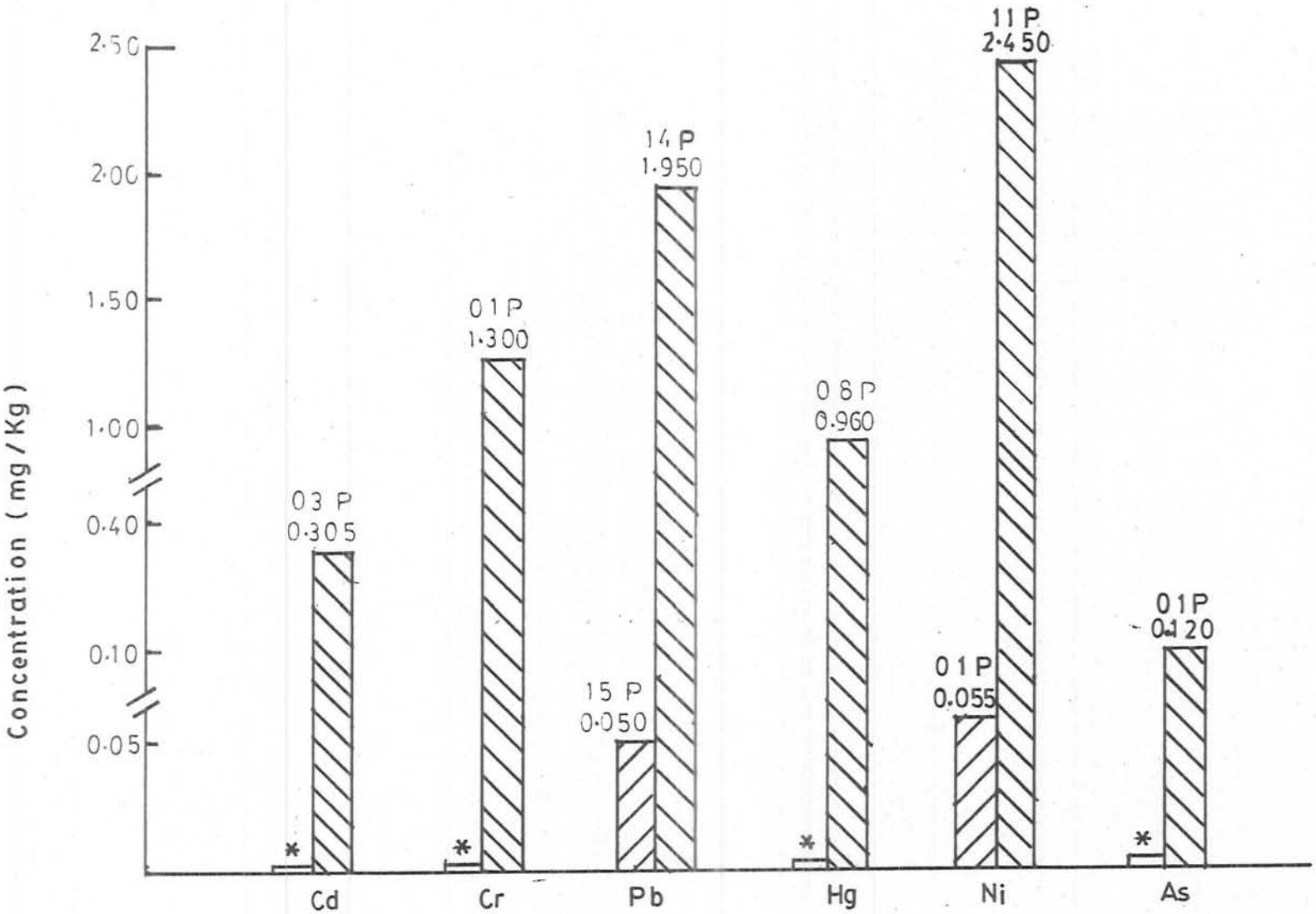



Fig. 4.2 Maximum and minimum levels of micronutrients in various grains ( sample code P) from Punjab




Fig· 4·3 Maximum and minimum levels (* for below detection limit) of toxic metals in various grains (sample code P) from Punjab

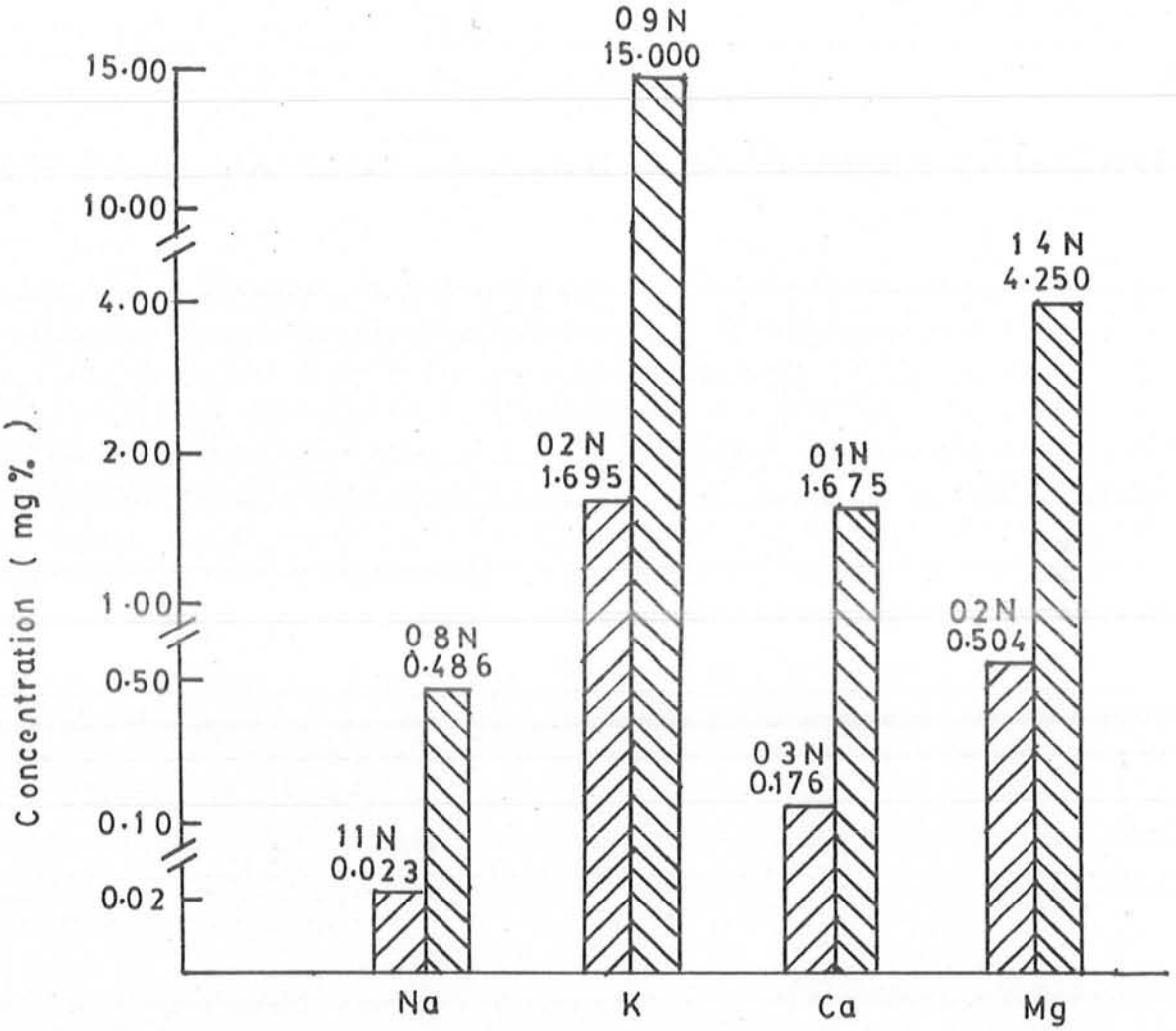



Fig 4.4 Maximum and minimum levels of macronutrients in various grains (sample code N) from NWFP

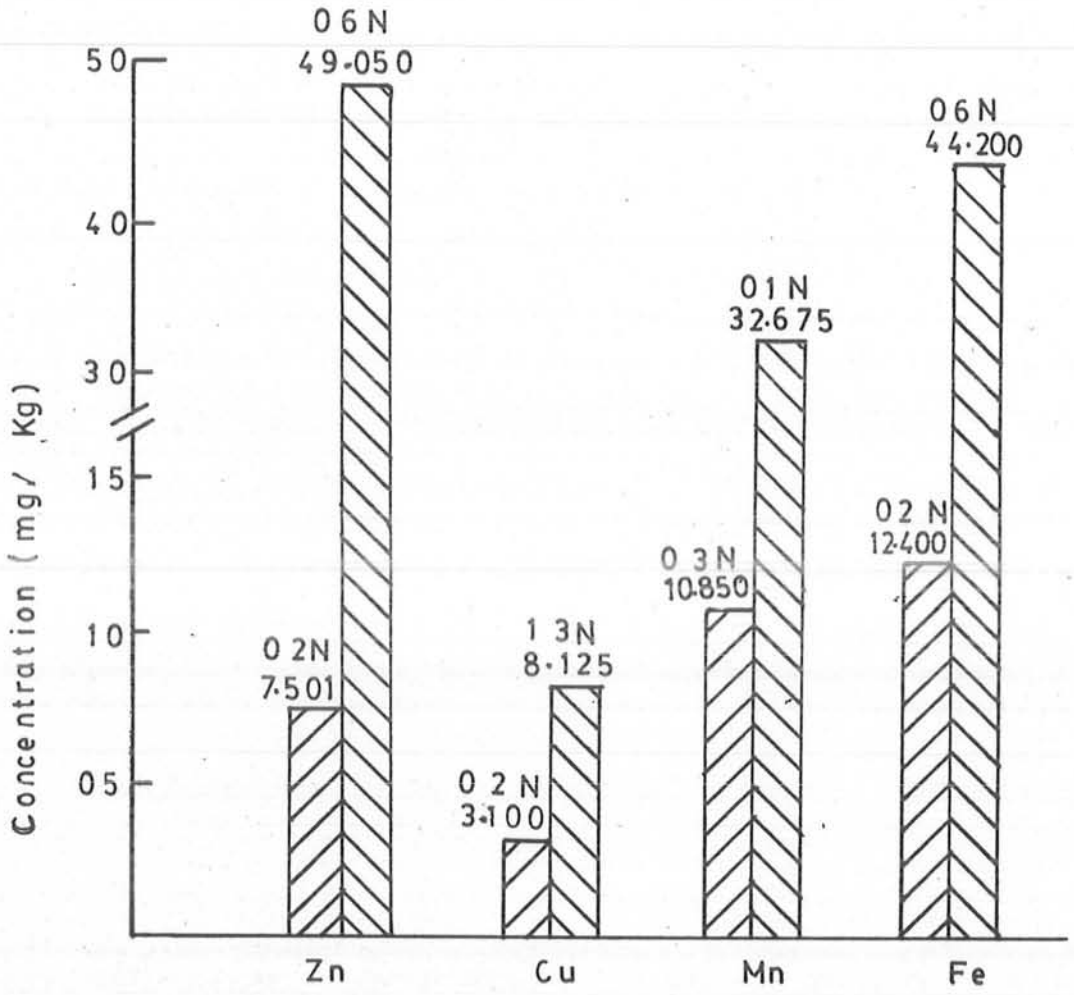



Fig 4.5 Maximum and minimum levels of micronutrients in various grains (sample code N) from NWFP

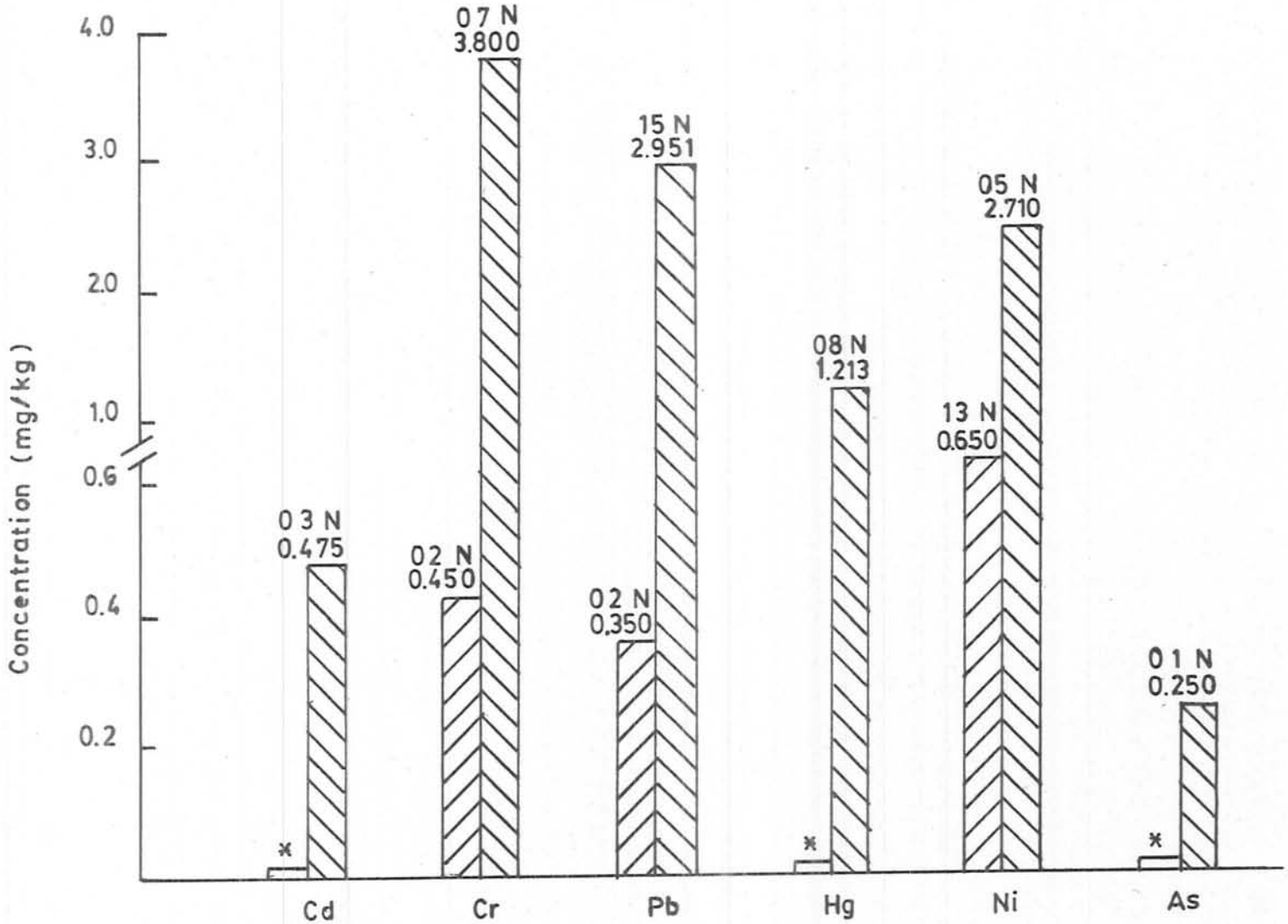



Fig. 4.6 Maximum and mimum levels (* for below detection limit) of toxic metals in various grain (sample code N) from NWFP

## 4.3 Discussion

The description of samples analysed during the present investigation for macro and micro trace metal levels, along with codes used, is given in Table 4.1. The subsequently tables (Table 4.2-4.7) include data on macro and micro nutrients together with toxic metals for various commodities of grains grown in the two provinces. Tables 4.8 and 4.9 list the macronutrient and micronutrient levels in various soil samples belonging to the origin of certain grains. Tables 4.10 and 4.11 embody the concentration of toxic trace metals in various soil samples and the organic matter content and pH respectively. The figures 4.1 to 4.6 pertain to histograms representing the extrimum levels of various grains from both the provinces. The entire data are represented on average basis computed for triplicate measurements in each case and expressed at ± s statistical level with an over all accuracy of about ± 1%. The reproducibility in individual cases was found to be better than less than 1%.

In all fifteen different food grains subjected to the present investigation and fourteen metals including the macro, micro and toxic elements, were estimated by atomicabsorption method. The relevant limits of detection for

the individual metal along with optimum operational parameters are given in Table 2.1.

sodium, potassium, calcium and magnesium were treated as essential macronutrients in all the food grains, while zinc, copper, manganese, and iron were taken as essential trace metals. In the list of toxic heavy trace metals, cadmium, chromium, lead, mercury, nickel and arsenic were included.

The macronutrients (K, Na, Ca, Mg) levels found in various grains were found to be quite divergent: for example, the potassium levels (Table 4.2) varied between 1.750 mg% to 14.625 mg% in samples 02P and 08P, both from Punjab, respectively. Similarly the sodium contents varied between 0.017 mg% to 0.408 mg%, for sample 09P and 04P respectively. The case of calcium showed less divergence and it was found that the minimum and maximum levels of Ca ranged between 0.089- 0,876 mg% in samples 02P and 01P respectively. A better situation evolved in case of magnesium where the minimum and maximum levels were present with a ratio of about 1:2.5. Thus, sample 02P representing rice, from Punjab has the minimum Ca, Mg and K levels and as such lacks the macronutrients. Sample 08P representing bean (white) from Punjab has the maximum K content at 14.625 mg% and therefore could be considered as a potassium rich food commodity.

Similarly sample 14P (mash whole) was found to be the best magnesium source with 4.500 mg% Mg content. On the same lines it was inferred that samples 01P and 04P, representing wheat and barley from Punjab are the best sources of sodium and calcium, estimated at 0.408 and 0.876 mg%. On the whole, the macronutrient levels estimated in these commodities fall within the estimated ranges of concentrations quoted in literature for these commodities. It was reported that the potassium content of US wheat[41] ranged between 4.56-5.82 mg% , as compared with our level at 5.21 ± 0.048 mg%. For the same wheat the reported Mg level ranged between 1.82-2.02 mg%, while our wheat samples had the corresponding range between 0.357- 4.500 mg% . The reported data[82] on the sodium content of wheat stands at 0.05 mg% , while in the present investigation the estimated range for sodium stood at 0.062 mg%. Thus, a close agreement existed between our estimated values and the quoted values in literature for wheat. This is not only specifically true about wheat but also for other commodities, noticeably for rice, maize and barley.

In the case of rice, the K, Mg, Ca and Na levels for samples from Punjab were estimated at 1.750, 0.357, 0.089 and 0.030 mg% respectively. All these levels are again in good agreement with the literature values reported by various

workers[83-86].

The relevant grain samples from NWFP yielded comparable results. As in the case of Punjab samples, the macronutrient levels in various grains from NWFP were found to be quite divergent, e.g., in the case of K, the extrimum values (Fig. 4.4) ranged between 1.695-15.000, for Mg 0.504-4.250, for Ca 0.176-1.675 and for Na 0.023-0.486 mg%. A direct comparison of these ranges with those found in literature[41,83-85,90-92] validated the current finding in terms of known international levels. On comparative grounds the Na content in the Punjab barley is maximum, while the Na content in NWFP bean (white) is maximum. A comparable situation for K in the Punjab bean (white) and NWFP mong (washed) also existed; same being true about Ca and Mg having ascending levels for wheat and rice. The province wise macronutrient situation for wheat and rice was therefore found to be very satisfactory. Although, various factors are responsible for the uptake of macronutrients by various food grains, yet the role of type of fertilizer used and its mode of application needed thorough investigation. This aspect required a temporal study extending over years and thus, the present investigation was restricted to estimating the macronutrient content for related grain sample along with the soil samples taken both

from Punjab and NWFP.

Four micronutrients (Zn, Cu, Mn, Fe) were estimated in various grains taken from Punjab and NWFP. The Zn content was found to be minimum in dal chana from Punjab and maximum in bean (red), at 14.750 and 37.450 mg/kg, dry weight basis. The range for Cu was 0.819-8.250 mg/kg for rice and mong (washed and whole). Similarly in case of Mn and Fe the corresponding levels estimated were 7.850 mg/kg ( maize from Punjab) -31.500 mg/kg (wheat from Punjab) and 4.400 mg/kg (rice from Punjab) -42.251 mg/kg (mash whole form Punjab) respectively. The NWFP grains were also found to be comparably rich in terms of these essential trace elements. The observed ranges were : Zn 7.501-49.050 mg/kg, Cu 3.100-8.125 mg/kg, Mn 10.850-32.675 mg/kg, Fe 12.400-44.200 mg/kg. These ranges were found in good agreement with corresponding commodity ranges represented by other workers[44,83-85,90-92]. Maximum Zn content was found in the Punjab bean (red) while, maximum Zn content was found in the black gram from NWFP. Maximum Cu content was found in mong (washed) from Punjab, and in mash (washed) from NWFP. The Punjab wheat had maximum Mn content as in the case of NWFP wheat. In the case of Fe its maximum content was found in mash (whole) from Punjab and in black gram from NWFP; the level being comparable, 42.251 against

44.200 mg/kg for the two provinces respectively. It was thus concluded that the essential trace metal levels in all the food grains belong to the two provinces were almost equally good from the view point of quality.

A proper attention was given during the present investigation to the estimation for toxic trace metals as well. In this category Cd, Cr, Pb, Hg, Ni and As were included. The mobilization of these toxic trace elements from soil into water en route to the plant is a specialized field of investigation, requiring thorough research on various aspects including the water and soil composition, pH, organic matter and uptake mechanism by relevant plants. As this required a prolonged time of investigation the present work was confined to the assessment of the soil quality in terms of its macronutrients, micronutrients and heavy trace metal counter part. Table 4.4 lists the levels of toxic trace metals in various grains from Punjab, and Table 4.6 provides the same informations for grains from NWFP. Figures 4.3 and 4.6 bring out a comparative evaluation of various food grains belonging to the two provinces on comparative bases. It turned out that the maize from Punjab and NWFP contained a comparable high level of Cd at 0.305 and 0.475 mg/kg respectively. For Cr the wheat of Punjab (01P) and the red

bean from NWFP (07N) were found to contain respectively the highest levels of the metal at 1.300 and 3.800 mg/kg. In the case of Pb the Punjab mash whole and the NWFP dal chana were found to contain the maximum levels of the metal. The Hg content was found to be exceptionally high in the Punjab and NWFP white bean. Apparently this indicated a sort of grain-specificity by bean for Hg. Logically under the same environmental conditions the uptake of Hg by other grains was not noticed. Nickel is well known as a toxic metal and unfortunately the metal had its maximum content at 2.450 mg/kg in washed masur from Punjab and at 2.710 mg/kg in white gram from NWFP; in the latter case the level exceeded the internationally laid down allowed limit of 2.5 mg/kg Ni in edible grains[93]. Lastly the As content was found to be at 0.120 mg/kg and 0.250 mg/kg, respectively in wheat of Punjab and NWFP. In view of the permissible limits emposed by international agencies in respect of the safe consumption of these food grains, it was observed that the local grains were quite free of excessive levels of heavy toxic trace metals. In most instances the lowest levels were found to be bellow the individual detection limit of the metal. In instances where higher levels were met with the origion of the heavy toxic trace metals could be traced as arising from either the

soil compositions or the natural water runoff from highly mineral rich soil terrains.

An investigation into the soil composition together with relevant parameters such as pH and organic matter content were quite revealing. The Punjab soil samples were found to be rich in Na, K, Ca and Mg contents, reflecting their enrichment in the food grains. On the whole, the Punjab and NWFP soils had comparable macronutrient contents as were found in the case of individual food grains. In the case of micronutrients the Punjab soil was found to be less rich in respect of Zn, more rich in respect of Cu, equally rich in respect of Mn and more rich in respect of Fe. Apparently, this appeared to be an obvious contradiction to the existing situation related to the mineral content of the two soils. The Punjab soil has natural chemical ingredients that are transported and mobilized in the plant segments at high organic matter content originating from the use of natural fertilizers. It is well known that the trace metal uptake by the plants is not a sole function of the trace metal content of the soil, rather it depends on the pH and the ambient soil conditions. The heavy trace metal content analysis of the two soils showed almost identical levels for the various heavy trace metal in the water extract of all the samples. Thus it

was envisaged only the soluble portion of the heavy metals (mostly the inorganic ionic forms) is readily absorbed by the plants. Hence, the picture on organic matter content could be only viably explained under this condition. The organic matter in the Punjab soils was found to be higher than NWFP soils. Although the present data are limited to a small number, yet their are indications that their is excess humus material in the Punjab soils. Perhaps this could be a contributing reason to the corresponding pH values for the soils. Normally a pH range of 7.5 to about 8.5 is considered to be most congenial for agricultural produce, the observed pH ranges for the Punjab and NWFP soils were found to be within close proximity to the above range.

In conclusion, the present investigation on the micro-, macronutrient as well as heavy toxic trace metals revealed that the local food grains contain the required stipulated levels of essential elements and do not contain excessive amounts of toxic trace elements, except for a few commodities. The investigation provided base line information on the quality of these grains and could be viably used for future quality control and measurements. However, there is a dire need to takeup this investigation on temporal bases so that a qualitative relationship could be stabilised between

trace metal uptake and composition in soil under various seasonal and regional conditions.

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