

**ENRICHMENT OF VEGETABLES WITH TRACE
MINERALS THAT MAY POTENTIALLY ACT AS
HYPOGLYCEMIC AGENTS IN THE
TREATMENT OF DIABETES**



By

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**INSTITUTE OF CHEMICAL SCIENCES,
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JAVED ABBAS BANGASH

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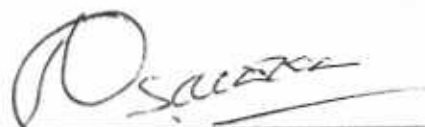
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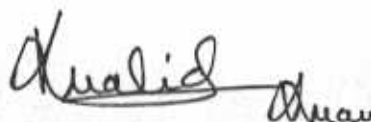
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DEDICATION

*Dedicated to
My elder sister
Nasreen*

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SUMMARY

Mineral elements must be acquired from the external environment, and because plants are non-mobile organisms, any minerals that a plant might acquire must be present in the local soil volume that can be reached by its root system.

With a well-balanced diet that includes mixed sources of grains, fruits and vegetables, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle. Fruits and vegetables are significant sources of RDA nutrients as well as other components that promote health. Vegetables are among the numerous plant adjuncts tried for the treatment of the diabetes mellitus. Some minerals, particularly the trace minerals are active participants of metabolism. Some of the minerals are the essential part of some of the enzymes for their biological activities. As diabetes mellitus is a disease of metabolic abnormality so minerals as such or as a component of enzyme may be playing a significant role in development and control of diabetes mellitus.

Seven vegetables from winter and six from summer were included in the present study to determine some of their mineral components and see if some of their mineral (Cr, Zn, Mn, Cu, Mg and Fe) content could be increased by supplementation through their roots. The winter vegetables were; Radish (*Raphanus-sativus*), Turnip (*Brassica-rapa*), Spinach (*Spinacea-oleracea*), Fenugreek/ Methi (*Trigonella-foenum-graceu*), Sarson (*Brassica-campestris-var-sarson*), Pot purslane (*Portulaca-oleracea*) and Garlic (*Allium-sativum*). Similarly summer season vegetables were; Bitter gourd (*Momordica-charantia*), Bath sponge (*Luffa acutangula*), Bringal/Egg-plant (*Solanum-melongena*), Pot-purslane (*Portulaca-oleracea*), Spinach (*Spinacea-oleracea*) , Ladyfinger local and Indian variety (*Hibiscus-esculentus*).

Fresh and healthy seeds were purchased from local market for winter and summer vegetables. Before sowing seeds, the soil was cultivated to a depth of more than one foot, removed stones and broken large clods until it had smooth and fine texture. A plot was prepared and divided into three columns marked as boxes (Box-A, Box-B and Box-C), each box further divided in to four sub-boxes (A-1, A-2, A-3, A-4) (B-5, B-6, B-7, B-8) and (C-9, C-10, C-11, C-12) respectively. Thus one row had

twelve sub-boxes and sub-box (C-12) was kept as control. Before sowing seeds, soil samples were taken and analyzed for soil pH, electrical conductivity (EC), texture, organic matter, lime content (CaCO_3), Potassium, Phosphorus and extractable heavy metals (Cr, Zn, Mn, Cu, Mg and Fe).

The seeds were planted and fertilizer Diammonium phosphate (DAP) was applied to the soil. 0.1Molar solution of each Chromium (III)-chloride, Zinc Sulfate, Manganese Sulphate, Copper Sulfate, Magnesium Sulfate and Ferrous Sulfate salts was prepared and applied to the roots of vegetables as individual or in combinations.

Among winter vegetables, Spinach (*Spinacea-oleracea*), Fenugreek/ Methi (*Trigonella-foenum-graceum*), Sarson (*Brassica-campestris-var-sarson*), Pot purslane (*Portulaca-oleracea*) were first supplemented after 10 days, Radish (*Raphanus-sativus*), Turnip (*Brassica-rapa*) after 20 days and Garlic (*Allium-sativum*) after 30 days of sowing and second supplementation was done after 15, 30 and 45 days of first supplementation.

Similarly, summer vegetables, Pot purslane (*Portulaca-oleracea*), Spinach (*Spinacea-oleracea*) were first supplemented after 13 days and Bitter gourd (*Momordica-charantia*), Bath sponge (*Luffa acutangula*), Bringal/Egg-plant (*Solanum-melongena*), Ladyfinger local and Indian variety (*Hibiscus-esculentus*) after 18 days of sowing and second supplementation after 10 and 20 days of first supplementation.

After harvesting vegetables were dried, acid digested and analyzed for Cr, Mn, Zn, Cu and Fe and Mg on Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer.

In winter vegetables increase in minerals concentration milligram per Kilogram dry weight basis observed was for chromium; (*Portulaca-oleracea* 40> *Spinacea-oleracea* 20> *Brassica-rapa* Roots 18>*Brassica-rapa* Leaves16> *Allium-sativum* 14 > *Brassica-campestris-var-sarson* 12> *Trigonella-foenum-graceum* 10> *Raphanus-sativus* Leaves and Roots 6). for zinc; *Portulaca-oleracea* 228> *Trigonella-foenum-graceum* 94> *Brassica-rapa* Roots 68> *Spinacea-oleracea* 66> *Brassica-rapa* Leaves 64> *Raphanus-sativus* Roots 62> *Raphanus-*

sativus Leaves 44> *Brassica-campestris-var-sarson* 30> *Allium-sativum* 28.for manganese; *Spinacea-oleracea* 32> *Brassica-campestris-var-sarson* 22> *Portulaca-oleracea* 20> *Trigonella-foenum-graceum* 10> *Allium-sativum* 4.for copper; *Raphanus-sativus* Leaves 32> *Raphanus-sativus* Roots 24> *Portulaca-oleracea* 8> *Brassica-rapa* Leaves 6> *Brassica-rapa* Roots and *Allium-sativum* 4. for magnesium; *Portulaca-oleracea* 9460> *Spinacea-oleracea* 1720> *Brassica-campestris-var-sarson* 424> *Trigonella-foenum-graceum* 256> *Raphanus-sativus* Roots 212> *Allium-sativum* 116.for iron; *Portulaca-oleracea* 684> *Trigonella-foenum-graceum* 52> *Spinacea-oleracea* 374> *Brassica-rapa* Roots 188> *Brassica-rapa* Leaves 72> *Allium-sativum* 8.

In summer vegetables total increase in minerals concentration milligram per Kilogram dry weight basis observed was for chromium *Momordica-charantia* 90> *Spinacea-oleracea* 90> *Hibiscus-esculentus* local variety34> *Hibiscus-esculentus* Indian variety24> *Luffa acutangula* 14> *Portulaca-oleracea* 12> *Solanum-melongena* 4. for zinc; *Spinacea-oleracea* 236> *Hibiscus-esculentus* Indian variety110> *Solanum-melongena* 108> *Luffa acutangula* 104> *Portulaca-oleracea* 62> *Momordica-charantia* 52> *Hibiscus-esculentus* local variety 36. for manganese; *Momordica-charantia* 32> *Portulaca-oleracea* 26> *Hibiscus-esculentus* Indian variety 14> *Luffa acutangula*; *Solanum-melongena* and *Hibiscus-esculentus* local variety 10. for copper; *Momordica-charantia* 40> *Luffa acutangula* and *Portulaca-oleracea* 20> *Solanum-melongena* 12> *Spinacea-oleracea* 8> *Hibiscus-esculentus* local variety and *Hibiscus-esculentus* Indian variety 6. for magnesium; *Spinacea-oleracea* 8800> *Luffa acutangula* 962> *Portulaca-oleracea* 660> *Hibiscus-esculentus* Indian variety 608> *Hibiscus-esculentus* local variety 486> *Momordica-charantia* 176> *Solanum-melongena* 134. for iron; *Spinacea-oleracea* 1142> *Momordica-charantia* 508> *Hibiscus-esculentus* local variety 88> *Solanum-melongena* 56> *Portulaca-oleracea* 24> *Hibiscus-esculentus* Indian variety 20.

From results of chromium and zinc of both winter and summer season vegetables, it is evident that by increasing soil concentration of these elements, their concentration could be increased to a significant level. The results obtained of both winter and summer vegetables for magnesium indicates that increasing magnesium concentration has no effect in increasing its concentration. The reason is, that

magnesium is a macro element and present in appreciable amount in the soil and also supplied in reasonable amount through water. From our results magnesium content was enhanced in the samples where chromium and zinc were applied as individual or in combinations. Thus chromium and zinc supplementation could have good effect on the absorption of magnesium. Maximum iron concentration was recorded on chromium supplementation. It seems as if chromium has synergistic effect on the uptake of iron.

Overall in winter and summer vegetables, where copper and zinc was supplemented, manganese and iron concentration was decreased. This indicates that high concentrations of copper or zinc or both of soil may inhibit iron and manganese.

To study the effect of plant maturity on the uptake of these metals, the supplementation was made in different intervals of plant maturity stages. Supplementation at first stage is best one for minerals uptake.

To study seasonal effects, two vegetables namely, Spinach (*Spinacea-oleracea*) and Pot purslane/Kolfa (*Portulaca-oleracea*) were sown both in winter and summer season. In case of *Spinacea-oleracea* chromium and manganese content was high in summer while Zn, Cu, Mg and Fe content was high in winter vegetables. Chromium, zinc, copper, magnesium and iron contents were increased in all summer vegetables. The increase is quite marked suggesting that summer season is more suitable for enhancing the contents of these minerals than winter. Similarly, the manganese content of control sample of summer is too high, and no further increase occurred even after supplementation. In case of *Portulaca-oleracea* comparing control results, chromium and iron content was higher in winter vegetables, while Zn, Mn, Cu and Mg content was higher in summer vegetables. Enhancement of chromium and iron content is also greater in winter than summer season. Another important finding is marked increase in zinc, magnesium and iron contents after supplementation. Zinc, Manganese, copper, and magnesium content was increased in all summer vegetables. The results suggest that for enhancing chromium, magnesium and iron contents, winter season is best. Similarly, for Zinc, Manganese, copper, and magnesium summer season is best.

To study variety effects, two varieties of *Hibiscus-esculentus* local and Indian were grown in the summer season to observe the minerals absorption capability. The results obtained from different varieties of same plant showed different tendency and capability to assimilate the minerals. The behavior towards supplementation of minerals was different.

From the present study it can be concluded that by changing the soil minerals environment the uptake of required mineral content of vegetables, perhaps could be enhanced. The maximum minerals absorption and increase was manifested by vegetables at first supplementation stage. Summer season remained more favorable on the basis of percent increase of minerals than winter season. Soil type, seasonal effects and plant variety have important effect on plant minerals absorption. Thus it is possible that with some other soil type and seasonal conditions the better results could be achieved. The present study has been especially correlated with diabetes management/control on the basis of provision of enhanced level of minerals through vegetables, which can play important role in diabetes and also in other deficiency diseases like anemia etc. As almost all vegetables are easily available, thus enhancing their mineral content by supplementation would be a good source for eliminating deficiency diseases.

CHAPTER-1

INTRODUCTION

The words enrichment and fortification have historical origins. Enrichment was originally introduced in the 1940s with enactment of the Standards for Enrichment Programs aimed at replenishing nutrients lost during cereal processing. This was expanded into a broader context to include nutrients not naturally present in the food, or fortification. Currently, the two words are often used interchangeably, which is wrong from a historical standpoint. However, taking into consideration the fact that the aim in both cases is to improve the nutritional value of foods, the term nutrification was suggested, which would include both enrichment and fortification (Bauernfeind, 1991).

All people are dependent on food systems of varying complexity to supply the nutrients required to sustain human life. Yet, food systems have developed with no attention given to their ability to provide all the nutrients required supporting good health, productive lives and well being. Inadequate nutrition lowers the quality of life for billions of disadvantaged people globally especially in many developing countries. Food systems should be exploited in ways that will improve their potential to provide adequate nutrient output to offer the poor a reasonable chance for healthier and more productive lives (Combs et al, 1997). How can we increase vegetable consumption in places where the economy does not encourage it? This is only possible through policies intended to reduce the relative prices of vegetables, increased income, enhancement of micronutrient contents in vegetables, generating awareness among consumers of the role of vegetables in human health, and improvements in vegetable cooking and processing methods (Mubarik and Tsou, 1997).

ENHANCING MINERAL CONTENT IN PLANTS

Plants are known to accumulate various chemicals from their environment (Singh and Narwal, 1984; Griffith et al., 2001). Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well-being (Linder, 1991). With a well-balanced diet that includes mixed sources of grains, fruits and

vegetables, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle (Dwyer, 1991).

Unfortunately, consumption patterns are not always ideal, and many individuals both in developed and developing countries are failing to attain recommended mineral intakes (Kennedy, 1997 and Bates, 1999). Whereas an increased consumption of plant food products would be beneficial, it appears that behavioral and/or environmental factors will continue to limit their consumption (Baranowski, 1997 and Baranowski, 1999). Thus, as an alternative strategy, efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals (Bouis, 1996 and Farnham, 1999).

Currently, the achievable densities of minerals in our existing agricultural crops mean that few individual plant foods are able to supply the daily recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains and tuber crops, which make up a large proportion of daily food intake in the developing world (Calloway, 1995).

Fortunately, plant mineral concentrations do vary both among plant sources i.e., species, cultivars and among plant tissues (e.g. leafy structures *versus* seeds) (Quintana, 1996 and Abbo, 2003), thereby demonstrating that genetic differences exist, which can contribute to the plant's ability to acquire and sequester minerals. Plant scientists are currently working to decipher the molecular components and physiological processes that underlie plant mineral nutrition, such that this understanding can be used to enhance the mineral content of agronomically important crop species. These efforts include classical breeding approaches designed to characterize and exploit genetic variation for mineral content (Bouis, 1996; Ruel, 1998 and Lin, 1997), as well as new approaches involving gene discovery and directed genetic modification to create new plant lines with improved mineral qualities (Grusak, 1999; DellaPenna, 1999 and Mori, 2001).

When designing transgenic approaches to enhance plant quality traits, there often is a tendency to look for one gene that will help complete a biochemical pathway and thereby serve to synthesize a nutrient of interest (DellaPenna, 1999 and Grusak, 1999). Of

course, for mineral enhancements, no gene products exist which allow a plant (or any other organism) to synthesize minerals. All mineral elements must be acquired from the external environment, and because plants are non-mobile organisms, any minerals that a plant might acquire must be present in the local soil volume that can be reached by its root system. If the mineral occurs in a soluble form (usually as an ionized inorganic ion) it potentially can be absorbed into the plant and be mobilized from the roots to various vegetative and reproductive tissues.

MINERALS UPTAKE BY PLANTS

Mineral elements, those acquired primarily in the form of inorganic ions, continually cycle through all organisms and their environment. The entry point of mineral elements to the biosphere is predominantly through the root systems of plants so that, in a sense, plants act as the "miners" of the earth's crust (Epstein, 1994).

The study of how plants absorb and assimilate inorganic ions is called mineral nutrition. This area is central to modern agriculture and environmental protection. High agricultural yields depend strongly on fertilization with mineral elements. In fact, yields of most crop plants increase linearly with the amount of fertilizer that they absorb (Loomis, 1992).

Plants have developed several biochemical processes for the mobilization and uptake of minerals. To chelate and solubilize soil minerals, the roots secrete metal-chelating molecules such as phytosiderophores (Romheld, 1991). Metal-chelating proteins such as metallothioneins (Robinson, et al, 1993) or phytochelatins (Rauser, 1990) also function in plants to bind metals and enhance transport and metabolism organic acids have also been implicated in metal transport (Baker, 1989).

Which minerals can plants absorb, and thus provide in the diet? An obvious initial list includes the 14 mineral elements defined as essential for plant growth and reproductive success (Marschner, 1995). These are N, S, P, K, Ca, Mg, Cl, Fe, Zn, Mn, Cu, B, Mo, and Ni. Because of their essentiality, all plant foods contain some level of each of these elements, and it should come as no surprise that plants have developed various forms of molecular machinery (i.e. membrane transporters) to acquire these

mineral nutrients from their soil environment (Kochian, 1991 and Maathuis, 2002). Of these 14 elements, human essentiality has been confirmed for all but B and Ni, although circumstantial evidence for their essentiality has been reported (Nielsen, 1996). Na, Cr, I and Se also are required by humans, but not by plants. Fortunately for humans, however, plants can acquire these other elements through non-specific influx processes using existing transporters localized to their roots (Kabata-Pendias, 1992). The overall uptake of these plant non-essential elements depends on their availability in the soil, in conjunction with the extent of their influx through non-specific transporters. In fact, a wide range of plant non-essential elements (both benign and detrimental) have been measured in plant tissues, with concentrations sometimes-reaching dramatic levels if soil availability is high (e.g. Cr, Se). A number of these elements, also referred to as the ultra trace elements, have been demonstrated to provide various health benefits in humans (Nielsen, 1996) and thus their incorporation into plant tissues is of dietary relevance.

FACTORS THAT CONTROL PLANT MINERAL CONTENT

Assuming a mineral ion is available in the soil, an appropriate transport protein must be present to facilitate its movement into the root symplasm. Once it enters this compartment, the mineral either can provide for the local nutritional needs of the root cells, or it can be moved to the xylem pathway. The xylem transport system is composed of columns of non-living cells (i.e. devoid of membranes) that provide an open route for the bulk flow of water and mineral ions (Kramer, 1995). This solution (xylem sap) moves up the plant in response to a reduction in water potential that is created primarily by the evaporative loss of water (i.e. transpiration) from various exposed structures. Most water leaves these structures through small pores known as stomata. Thus, minerals within the xylem sap are delivered non-selectively as columns of water are pulled up through the plant and are accumulated preferentially in those tissues with high rates of water loss. These tissues include most leaves and certain reproductive structures, such as the pod walls of legume species or the leaf-like organs encompassing certain cereal grains (e.g. rice, wheat). It should be noted that non-exposed structures (e.g. most developing seeds, closely packed leaves) or organs with low densities of stomatal pores (e.g. many fruit) can exhibit very low, or even no rate of transpiration (Kramer, 1995 & Grusak, 1999). Consequently, mineral delivery via the xylem pathway to these types of organs will be low to non-existent (Tibbitts, 1979).

How do these poorly transpiring structures get minerals? A second long-distance pathway, known as the phloem transport system, is responsible for the movement of sugars, amino acids, certain minerals and various secondary metabolites from photosynthetic "source" organs (primarily leaves) to several "sink" regions (Marschner, 1995). Sinks include such things as reproductive tissues (seeds, fruit), young developing leaves and roots. The phloem transport pathway is composed of columns of cells, but unlike the situation in the xylem pathway, these are living cells bounded by membranes. Phloem sap moves from cell to cell through membrane-lined pores; the driving force is a hydrostatic pressure gradient which is established as solutes enter the phloem symplasm in source regions (causing an osmotic influx of water) and exit the symplasm in sink regions (with a contrasting osmotic efflux of water). Solute movement into the phloem symplasm can occur either via membrane transporters or through symplastic connections between phloem and surrounding cells (Grusak, 1996). In either case, the predominant solute in phloem sap is a sugar molecule (usually sucrose, but it could be various oligosaccharides), and except for potassium, most minerals are minor solutes in this system (Ziegler, 1975).

FOOD MINERALS VERSUS NON-FOOD MINERALS

The rapid growth of the mineral supplement industry is in part due to the need for supplements in diets lacking sufficient mineral content, but supplements may not provide minerals in a soluble and metabolically available form (Fairweather-Tait, 1996).

The most challenging aspect of providing trace elements in a plant-based material is to obtain a sufficient concentration for the supplement to be ingested without consuming large quantities of plant tissue. The use of forage crops enriched in Se from Se enriched soils to supplement the diets of animals has been proposed (Banuelos, et al, 1997).

SOIL FERTILITY AND HUMAN HEALTH

The importance of minerals in the soil and their effects on human health are not new concepts. Dr. Alexis Carrel, Winner of the Nobel Prize in Medicine in 1912, states,

“Soil is the basis of all human life and our only hope for a healthy world.... All of life will be either healthy or unhealthy according to the fertility of the soil. Minerals in the soil control the metabolism of cells in plant, animal and man. Variations in the distribution of certain minerals in the environment are known to have an effect on health (Ed. Charles, 1989).

The only problem is that over the years humans have become victims of the water cycle. Soil erosion leads to the depletion of essential nutrient elements in crops grown in depleted soils. When people consume a diet derived from such crops, the intake of essential elements becomes inadequate. This leads to the impairment of the relevant physiological functions, and causes disease” (Aswathanarayana, 1994).

Minerals work in combination with each other and with other nutrients, so imbalances of any mineral can cause health problems – too little of any essential mineral can lead to deficiency diseases, and too much of any mineral can be toxic (Shils , 1999).

We get these essential minerals primarily through the foods we eat. Good sources of essential minerals include fruits, vegetables, meats, nuts, beans and dairy products. Unfortunately, much of the soil in which food is grown has been depleted of these nutritive minerals; therefore the mineral content in food is reduced (Wauchope, 1978 and Lappalainen, 1981). We also obtain some minerals from the water we drink, but the amounts vary widely.

WHAT IS DIABETES?

Diabetes mellitus is a syndrome with disordered metabolism and inappropriate hyperglycemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate.

Type-1 Diabetes Mellitus: This type is due to pancreatic islet β - cell destruction by an autoimmune process. This severe form is usually associated with ketosis in its untreated state. It occurs most commonly in juvenile but occasionally in adults, especially non-obese and those who are elderly when hyperglycemia first appears. It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated and the pancreatic β - cell fail to respond to all insulinogenic stimuli. Exogenous insulin is

therefore required to reverse the catabolic state, prevent ketosis and reduce the elevated glucagon and blood levels. Certain human leukocyte antigens (HLA) are strongly associated with the development of type-1 diabetes.

Type-2 Diabetes Mellitus: This represent a heterogeneous group comprising milder forms of diabetes that occur predominantly in adults but occasionally in juveniles. Circulating endogenous insulin is sufficient to prevent ketoacidosis but inadequate to prevent hyperglycemia in the face of increased needs owing to tissue insensitivity. This type is subdivided in to two subgroups by the absence of obesity.

Non-obese type-2 patients: These patients generally show an absent or blunted phase of insulin release in response to glucose. The response may be elicited in response to other insulinogenic stimuli such as acute intravenous administration of sulphonylurease, glucagon or secretin.

Obese type-2 patients: This form is secondary to extra pancreatic factors that produce insensitivity to insulin.

Without insulin, the patients with type-1 diabetes develop serious metabolic complications such as acute ketoacidosis and coma (Pizzorno and Murra, 1992). The discovery of insulin dramatically altered the treatment of diabetes so those patients seldom die of diabetic coma due to uncontrolled levels of glucose. However, their survival has allowed all the complications of diabetes to become apparent (Kelly, 1995). Diabetes mellitus is a global disease, found in all nations of the world. The world wide prevalence of diabetes mellitus is expected to double between 1994 and 2010 to more than 240 million people (Mc Carty and Zimmet, 1997).

Non-insulin dependent diabetes mellitus (NIDDM) or Type-2 diabetes is one of the largest health problems in Western countries. NIDDM currently affects some 612 million persons in the U.S. and incidence is increasing despite continual advances in medical care. Approximately 95% of diabetics are Type-2 presenting with symptoms well into adulthood. While there is some genetic propensity for development of NIDDM, the etiology of this disease is largely nutritional. The small numbers of genetic factors

currently identified with NIDDM are likely to be factors which are expressed by current Western diets (Feskens, 1992 and Szathmary, 1994).

No genes particular to NIDDM have thus far been identified, but there are combinations of candidate genes which may confer susceptibility toward glucose intolerance and insulin resistance (Elbein, 1997). However, these various genetic factors may not have led to NIDDM in the past or under other circumstances. It is crucial to understand that unlike Type-1 (insulin-dependent diabetes), NIDDM is almost entirely both caused and prevented by nutritional choices, thus it is amenable to holistic alternative medical treatment.

Broad Hurst (Broad Hurst, 1997) proposed the following list, namely that the major causative factors for NIDDM, while exacerbated by aging, can all be related to a modern agricultural-processed food based diet:

1. Obesity and over fatness: In particular, simultaneously increasing lean body mass with resistance training while decreasing body fat provides significant improvement in glycemic control.
2. Carbohydrate (especially in refined and processed forms) and fat over nutrition.
3. Lack of polyunsaturated fatty acids (PUFA) in plasma membranes and unbalanced triglyceride intake.
4. Chromium deficiency.
5. Lack of soluble fiber and relevant beneficial phytochemicals.

DIABETES AND ITS TREATMENT

Diabetes mellitus is a major world health problem. Drug and diet therapies are the major approaches used in the treatment and control of the disease. Usually oral hypoglycemic drugs are used for the control of the disease. When hypoglycemic drugs are not working, then the treatment is shifted to insulin. Insulin therapy is successful in the beginning, but with course of time, insulin requirement increases. High doses of insulin are dangerous as high insulin circulation in the blood promotes diabetic complications and atherosclerosis.

The diet therapy approach is economical and feasible in developing countries. Diet therapy does not create the impression of being sick in diabetic individuals. This therapy consists basically of precautions concerning diet composition, the amount, distribution and timing of food intake (Camerini-Davalos, 1987). The main focus in the diet approach is on the adjustment of macro nutrients particularly the contents of carbohydrates and fats and restrictions on simple sugars in the diet. Carbohydrates and fats are important in the etiology and control of diabetes, but fruits, vegetables and other natural products may have insulin potentiating factor (IPF) which has a direct role in the prevention and control of the diabetes (Khan et al, 1993).

ROLE OF VEGETABLES IN THE TREATMENT OF DIABETES

Researchers have repeatedly observed health benefits associated with high fruit and vegetable consumption, it has not been possible to identify a specific constituent or, more likely, combinations of several constituents acting in concert that may be responsible for these benefits (Willett, 1998 and Schorah, 1999). The sources, which support the popular pharmacopoeia, are raw materials of vegetable, animal and mineral origin. However, the most important therapeutic resource is that of vegetable origin (Santana, 1996).

Vegetables are among the numerous plant adjuncts tried for the treatment of the diabetes mellitus. A few vegetables that are commonly consumed in India have been claimed to possess anti diabetic potency. In recent years, there has been a renewed interest to screen such plant food materials, for a possible beneficial use. Considerable

amount of work has been carried out in this regard with bitter gourd (*Momordica charantia*) (Kavikumar, 1997) and ivy gourd (*Coccinia indica*) (Kamble, 1996) both in experimental animals and human diabetic subjects. The limited number of studies on other vegetables such as cabbage (*brassica oleracia*), capsicum (*Capsicum annum*) (Monosereenusorn, 1980) green leafy vegetables, beans and tubers have shown the beneficial hypoglycemic influence in both experimental animals and humans. Other plants like *Memecylon umbellatum*, (Amalraj, 1988) *Eucalaptus globulus*, (Gray, 1988) *Agrimony eupatoria* (agrimony), *Agaricus campestris* (mushrooms), (Gray, 1998) *Caesalpinia bonducella* (leguminose), (Sharma , 1997) *Medicago sativa* (alfa alfa), (Gray, 1997) Basil leaves (Tinnart, 1983). In addition to its other complications, type-2 diabetes is associated with an increased risk of cardiovascular diseases, which are the leading cause of death in diabetic individuals. Although the evidence for a beneficial effect of a diet rich in fruits and vegetables on diabetes is not as strong as it is for heart disease, the results of a small number of studies suggest that higher intakes of fruits and vegetables are associated with improved control of blood glucose levels and lower risk of developing type-2 diabetes. In a cohort of almost 10,000 adults in the U.S., the risk of developing type-2 diabetes over the next 20 years was approximately 20% lower in those who reported consuming at least five servings/day of fruits and vegetables compared to those who reported consuming none (Ford, 2001).

Fruits and vegetables are significant sources of RDA nutrients as well as other components that promote health, often referred to as phytochemicals. Plants provide essential mineral and organic nutrients to humans either directly or indirectly when plants are consumed by animals, which are then consumed by humans (Grusak and Dellapenna, 1999).

There is significant genetic diversity for micronutrient uptake traits that can be used to enrich micronutrient elements in plants grown on micronutrient-poor soils (Marschner, 1995).

EFFECT OF MINERALS ON DIABETES MELLITUS

A large body of experimental evidence in animals and humans supports a significant role for minerals in diabetes mellitus (Baker, 1992 and Walter, 1991).

Minerals are essential component of life and hence of diets. They perform important functions in the various biochemical processes of the body. Some minerals, particularly the trace minerals are active participants of metabolism. Some of the minerals are the essential part of some of the enzymes for their biological activities. As diabetes mellitus is a disease of metabolic abnormality so minerals as such or as a component of enzyme may be playing a significant role in development and control of diabetes mellitus.

Amongst minerals, chromium is involved in the development and control of diabetes mellitus. Extensive research on the role of chromium in the development and control has been reported. Chromium is an essential micronutrient that functions in carbohydrate and lipid metabolism primarily via its role in the potentiation of insulin action. In the presence of chromium in a usable form, much lower levels of insulin are required (Anderson, 1993).

Direct association of trace and macro-elements in relation to human disease has been observed in many research studies. In many cases, an alteration in the metabolism of these minerals has been demonstrated (Prasad, 1998 and Nour Mohammadi, 1989).

Some trace elements act as antioxidants and prevent membrane per oxidation. Others act directly on glucose metabolism. Ceruloplasmin, the major plasma copper-transporting protein, possesses a potent antioxidant property. Chromium has been identified as an active component of the glucose tolerance factor (GTF). Manganese is a cofactor for a number of enzymatic systems including arginase which has been found to be elevated in diabetic animals such as rats and mice (Bond, 1983). Magnesium has an important role as a cofactor in the phosphorylation of glucose and in many other enzymatic reactions.

The function of zinc (Zn) in the body metabolism is based on its enzymatic affinity, way of a Zn-enzyme complex or Zn metalloenzyme. In humans and animals, diabetes results in disturbance of this vital trace element (Kinlaw, 1983).

Chromium is necessary for normal carbohydrate and lipid metabolism. It is believed to enhance insulin binding to cell receptors. Two-hundred microgram per day elemental chromium, from chromium picolinate, reduced the insulin requirement in

71.6% of 58 adults with type-1 diabetes, as compared to 74% of type-2 diabetics (Ravina, 1995).

Another study comparing the same form and dose on type-1 and type-2 diabetics found a greater difference between the effects on type-2 and type-1. Requirements for insulin or other hypoglycemic agents were reduced in 57.2% of type-2 diabetics compared to 33.6% of type-1 diabetics (Ravina, 1993).

Insulin dependent diabetes mellitus (IDDM) patients tend to be low in serum magnesium. A group of 71 people who had had diabetes for 10-20 years was divided into two subgroups depending on severity of retinopathy. Hypomagnesemia was most pronounced in patients with the most severe retinopathy (McNair, 1978). IDDM has been found to cause altered electrolyte metabolism and derangements in the parathyroid hormone-vitamin D axis, resulting in lower ionized calcium, magnesium, parathyroid hormone (PTH), calcitriol, and osteocalcin in children with IDDM. Supplementation of magnesium at 6mg/kg body weight in 23 IDDM children resulted in increased levels of calcium, magnesium, PTH, calcitriol, and osteocalcin (Saggese, 1991).

Tarui found diabetic patients exhibited elevated urinary zinc levels which were normalized with insulin treatment (Tarui, 1963). As mentioned previously, zinc may exert an insulin-like effect. Injections of zinc chloride to alloxan-induced diabetic rats prevented the hyperglycemia generally observed 24 hours after alloxan (Tadros, 1982). Zinc appears to protect islet cells from destruction as well as to enhance the effect of insulin when the two are given in tandem.

(Kosenko, 1968) found the mean manganese content of whole blood of diabetics to be one-half that of normal subjects. Like zinc, manganese administration also prevented the post-alloxan rise in blood glucose. In the case of manganese, however, the beta cells were not protected from destruction. In this same study, neither chromium nor cobalt protected rats from the diabetogenic effects of alloxan (Tadros, 1982).

CHROMIUM

Chromium (Cr) was discovered in 1797 by Vauquelin. It is a relatively common element, with a mean concentration in United States soils of about 40 mg/kg (Barnhart, 1997).

Although trivalent chromium is recognized as a nutritionally essential mineral, scientists are not yet certain exactly how it functions in the body. The two most common forms of chromium are trivalent chromium (III) and hexavalent chromium (VI). Chromium (III) is the principal form in foods, as well as the form utilized by the body. Chromium (VI) is derived from chromium (III) by heating at alkaline pH and is used as a source of chromium for industrial purposes. It is a strong irritant and is recognized as a carcinogen when inhaled. At low levels, chromium (VI) is readily reduced to chromium (III) by reducing substances in foods and the acidic environment of the stomach, which serve to prevent the ingestion of chromium (VI) (Stoecker, 1999 & Food and Nutrition Board, 2001).

The two common oxidation states of Cr present in the environment are Cr^{+3} and Cr^{+6} . The first one is considered to be the trace element essential for proper functioning of living organisms. While Cr^{+6} was reported to exert toxic effects on biological systems and is known as the most mobile Cr form in soil and water systems, whereas Cr^{+3} is generally not transported over great distances because of its low solubility and tendency to be adsorbed in the pH range typical for natural soils and waters. Hexavalent anionic form is more available for living organisms than is Cr^{+3} , and plays a main role in removing this metal from water and soil systems (Kotas, 2000). Chromium is one of the heavy metals that have nutritional importance (Kabata-Pendias and Pendias, 2001).

The main source of Cr in natural soils is weathering of their parent materials. The average amount of this element in worldwide soil surface is 54 ppm (Kabata-Pendias and Pendias, 2001).

DIETARY SOURCES OF CHROMIUM

The amount of chromium in foods is variable, and it has been measured accurately in relatively few foods. Presently, there is no large database for the chromium content of foods. Processed meats, whole grain products, ready-to-eat bran cereals, green beans, broccoli, and spices are relatively rich in chromium. Foods high in simple sugars, such as sucrose and fructose, are not only low in chromium but have been found to promote chromium loss (Lukaski, 1999).

Estimated average chromium intakes in the U.S. range from 23-29 mcg/day for adult women and 39-54 mcg/day for adult men (Food and Nutrition Board 2001).

Chromium is an essential dietary nutrient and is required for sugar and fat metabolism. Normal dietary intake of Cr^{+3} for humans is often insufficient with up to 90% of American diets deficient in chromium. In spite of chromium's relative abundance in soils, foods are generally low in chromium content. With the exception of herbs and condiments, and certain other special food items with a relatively low average consumption rate, such as tea, coffee and some candies, most foods contain Cr^{+3} below 100 micrograms/kg. Staple foods, particularly cereals and milk are very low (less than 10 micrograms/kg) in Cr^{+3} and show little or no geographic variation (Kumpulainen, 1992).

Good sources include Liver, American cheese, brewer's yeast, wheat germ, meat, fish, fruits, whole grains, brown sugar, alfalfa, and animal fats (Porter, 1999).

FUNCTION

A biologically active form of chromium participates in glucose metabolism by enhancing the effects of insulin. Insulin is secreted by specialized cells in the pancreas in response to increased blood glucose levels, for example, after a meal, insulin binds to insulin receptors on the surface of cells, activating those receptors and stimulating glucose uptake by cells. Through its interaction with insulin receptors, insulin provides cells with glucose for energy and prevents blood glucose levels from becoming elevated. In addition to its effects on carbohydrate (glucose) metabolism, insulin also influences the metabolism of fat and protein. A decreased response to insulin or decreased insulin

sensitivity may result in impaired glucose tolerance or type 2 diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes is characterized by elevated blood glucose levels and insulin resistance (Food and Nutrition Board 2001).

The precise structure of the biologically active form of chromium is not known. Recent research suggests that a low-molecular-weight chromium-binding substance (LMWCr) may enhance the response of the insulin receptor to insulin. First, the inactive form of the insulin receptor is converted to the active form by binding insulin. The binding of insulin by the insulin receptor stimulates the movement of chromium into the cell and results in binding of chromium to apoLMWCr, a form of the LMWCr that lacks chromium. Once it binds chromium the LMWCr binds to the insulin receptor and enhances its activity. The ability of the LMWCr to activate the insulin receptor is dependent on its chromium content. When insulin levels drop due to normalization of blood glucose levels, the LMWCr may be released from the cell in order to terminate its effects (Vincent, 2000a).

Investigations over the last two decades have suggested that there is a naturally occurring bio-molecule that binds trivalent Cr that can explain how Cr^{+3} is involved in carbohydrate and lipid metabolism. This molecule, chromodulin (originally termed low-molecular-weight Cr-binding substance), is a naturally occurring oligopeptide composed of glycine, cysteine, aspartate and glutamate with the carboxylates comprising more than half the total amino acid residues (Yamamoto et al. 1987; Davis & Vincent, 1997a).

Despite its small size (molecular weight approximately 1438 for the bovine liver material), the molecule tightly binds four equivalents of Cr^{+3} (Sun et-al. 2000).

Spectroscopic studies suggest that the Cr^{+3} comprise an anion-bridged multinuclear assembly supported by carboxylates from the oligopeptide (Davis & Vincent, 1997a; Jacquamet et al. 2003).

Chromodulin has been isolated from the liver or kidney of several mammals and purified. A related Cr-containing oligopeptide (M-low-molecular-weight Cr-binding substances) has been isolated from bovine colostrums, which comprises the same amino

acids, but in distinctly different ratios, and also stimulates insulin-dependant glucose metabolism in rat adipocytes (Yamamoto et al. 1988).

The relationship between the milk and liver oligopeptides is deserving of additional study and suggests the possibility that other carboxylate rich oligopeptides might exist and play a role in Cr transport or function. Chromodulin has been shown to activate the tyrosine kinase activity of insulin-activated insulin receptor (Davis et al. 1997; Davis & Vincent, 1997b) and to activate a membrane phospho-tyrosine phosphatase in adipocyte membranes (Davis et al. 1996). For example, the addition of bovine liver chromodulin to rat adipocytic membranes in the presence of 100 nM-Insulin results in an up to eight-fold stimulation of insulin-dependent protein tyrosine kinase activity that is concentration dependant, while no activation of kinase activity is observed in the absence of insulin (Davis & Vincent, 1997b). The dependence of the kinase activation on the concentration of chromodulin can be fitted to a hyperbolic curve to give dissociation constants of approximately 875 pM. Cr plays a crucial role in the activation of insulin receptor kinase activity by chromodulin (Davis & Vincent, 1997b). Apochromodulin does not activate insulin-dependant tyrosine kinase activity in rat adipocyte membranes. Titration of apochromodulin with Cr^{+3} results in the total restoration of the ability to activate kinase activity; approximately four Cr^{+3} per oligopeptide are required for maximal activity. This reconstitution of chromodulin's activation potential is specific to Cr; addition of other biologically relevant metals does not restore activity to the apo-oligopeptide. Based on these results, it has been proposed that chromodulin function as part of a unique auto amplification system for insulin signaling (Vincent, 2000b, c). In this mechanism apochromodulin is stored in insulin-sensitive cells. In response to increases in blood insulin concentrations insulin binds to its receptor, bringing about a conformation change that results in the auto-phosphorylation of tyrosine residues on the internal side of the receptor. This process transforms the receptor into an active tyrosine kinase and transmits the signal from insulin into the cell. In response to insulin Cr is moved from the blood to insulin-sensitive cells. Here, the Cr flux results in the loading of apochromodulin with Cr. The holochromodulin then binds to the receptor, presumably assisting in the maintenance of the receptor in its active conformation, amplifying the receptor's kinase activity. When the signaling is to be turned off, a drop in blood insulin levels facilitates relaxation of the conformation of the receptor, and the holochromodulin is excreted from the cell into the blood. Ultimately,

chromodulin is efficiently excreted in the urine. The Fe-transport protein transferring has recently been shown to be responsible for maintaining Cr^{+3} levels in the blood plasma and for transporting Cr to tissues in an insulin-responsive manner (Vincent, 2000a, Clodfelder et al. 2001).

Chromium is a component of a molecule called glucose-tolerance factor (GTF), which occurs naturally in the body and enhances the action of insulin at the cellular level (Toepfer, 1977).

The exact molecular structure of GTF is still unknown, and no one has been able to synthesize it. Therefore, claims that "GTF chromium" supplements are superior to other well-absorbed forms of chromium (such as chromium aspartate, chromium picolinate, or chromium polynicotinate) are questionable. A new study has found that dietary supplementation with chromium can moderate glucose intolerance and control blood sugar in diabetic patients (Rabinovitz, et al. 2004).

NUTRIENT INTERACTIONS

Iron: Chromium competes for one of the binding sites on the iron transport protein, transferrin. However, supplementation of older men with 925 mcg of chromium/day for 12 weeks did not significantly affect measures of iron nutritional status (Campbell, 1997). A study of younger men found an insignificant decrease in transferrin saturation with iron after supplementation of 200 mcg of chromium/day for 8 weeks, but no long-term studies have addressed this issue (Lukaski, 1996).

Vitamin C: Chromium uptake is enhanced in animals when given at the same time as vitamin -C. In a study of three women, administration of 100 mg of vitamin C together with 1 mg of chromium resulted in higher plasma levels of chromium than 1 mg of chromium without vitamin -C (Food and Nutrition Board 2001).

Carbohydrates: Diets high in simple sugars (e.g., sucrose), compared to diets high in complex carbohydrates (e.g., whole grains), increase urinary chromium excretion in adults. This effect may be related to increased insulin secretion in response to the

consumption of simple sugars compared to complex carbohydrates (Food and Nutrition Board 2001).

DEFICIENCY

Chromium deficiency was reported in three patients on long-term intravenous feeding who did not receive supplemental chromium in their intravenous solutions. These patients developed evidence of abnormal glucose utilization and increased insulin requirements that responded to chromium supplementation. Additionally, impaired glucose tolerance in malnourished infants responded to an oral dose of chromium chloride.

Several studies of male runners indicated that urinary chromium loss was increased by endurance exercise, suggesting that chromium needs may be greater in individuals who exercise regularly (Lukaski, 2000). In a more recent study, resistive exercise (weight lifting) was found to increase urinary excretion of chromium in older men. However, chromium absorption was also increased, leading to little or no net loss of chromium as a result of resistive exercise (Rubin, 1998).

At present, research on the effects of inadequate chromium intake and risk factors for chromium insufficiency are limited by the lack of sensitive and accurate tests for determining chromium nutritional status (Stoecker, 1999 & Food and Nutrition Board 2001).

DISEASE TREATMENT AND PREVENTION

Diabetes mellitus

In 12 out of 15 controlled studies of people with impaired glucose tolerance, chromium supplementation was found to improve some measure of glucose utilization or to have beneficial effects on blood lipid profiles (Kobla, 2000). Impaired glucose tolerance refers to a metabolic state between normal glucose regulation and overt diabetes. Generally, blood glucose levels are higher than normal, but lower than those accepted as diagnostic for diabetes. Impaired glucose tolerance is associated with

increased risk for cardiovascular diseases but is not associated with the other classic complications of diabetes. About 25- 30% of individuals with impaired glucose tolerance eventually develop type-2 diabetes (Goldman, 2000). Generally, chromium supplementation at doses of about 200 mcg/day in a variety of forms for two to three months was found to be beneficial. The reasons for the variation or lack of effect in some studies are not clear, but chromium depletion is not the only known cause of impaired glucose tolerance. Additionally, the lack of an accurate measure of chromium nutritional status prevents researchers from identifying those individuals who are most likely to benefit from chromium supplementation (Stoecker, 1999 and Anderson, 1998).

Type-2 diabetes is characterized by elevated blood glucose levels and insulin resistance. Although insulin levels in type-2 diabetics may be higher than in healthy individuals, the physiological effects of insulin are reduced. Because chromium is known to enhance the action of insulin, the relationship between chromium nutritional status and type-2 diabetes has generated considerable scientific interest. Individuals with type-2 diabetes have been found to have higher rates of urinary chromium loss than healthy individuals, especially those with diabetes of more than 2 years duration (Morris, 1999).

Chromium, a naturally occurring element obtained from food, is considered an essential trace element. Patients take this supplement for diabetes, weight loss, and to improve muscle mass. Chromium is poorly absorbed following oral administration, although the salt forms (e.g. chromium picolinate, niacin-bound chromium, and chromium chloride) appear to have better bioavailability. Chromium is believed necessary for glucose and lipid metabolism. It is involved with the activity of insulin (Porter, 1999 and Vincent, 2000).

OTHER DISEASES

Cardiovascular diseases

Impaired glucose tolerance and type-2 diabetes are associated with adverse changes in lipid profiles and increased risk of cardiovascular diseases. Studies examining the effects of chromium supplementation on lipid profiles have been notable for their inconsistent results. While some studies have observed reductions in serum total

cholesterol, LDL-cholesterol and triglyceride levels or increases in HDL-cholesterol levels, others have observed no effect. Such inconsistent responses of lipid and lipoprotein levels to chromium supplementation may reflect differences in chromium nutritional status. It is possible that only those individuals with insufficient chromium will experience beneficial effects on lipid profiles due to chromium supplementation (Stoecker, 1999; Lukaski, 1999 and Kobla, 2000).

Increases muscle mass: Chromium supplementation increases lean body mass and decreases body fat is based on the relationship between chromium and insulin action. In addition to affecting glucose metabolism, insulin is known to affect fat and protein metabolism. At least 12 placebo-controlled studies have compared the effect of chromium supplementation (200-1000 mcg as chromium picolinate/day) with or without an exercise program on lean body mass and measures of body fat. In general, those studies that have used the most sensitive and accurate methods of measuring body fat and lean mass (dual energy x-ray absorptiometry or DEXA and hydro densitometry or underwater weighing) do not indicate a beneficial effect of chromium supplementation on body composition (Lukaski, 1999 and Kobla, 2000).

Promotes weight loss: Controlled studies of chromium supplementation (200-400 mcg as chromium picolinate/day) have demonstrated little if any beneficial effect on weight or fat loss (Volpe, 2001), and claims of weight loss in humans appear to be exaggerated. In 1997 the U.S. Federal Trade Commission (FTC) ruled that there is no basis for claims that chromium picolinate promotes weight loss and fat loss in humans (Lukaski, 1999; Kobla, 2000 and Anderson, 1998).

SUPPLEMENTS

Chromium (III) is available as a supplement in several forms: chromium chloride, chromium nicotinate, chromium picolinate, and high-chromium yeast. They are available as stand-alone supplements or in combination products. Doses typically range from 50 to 200 mcg of elemental chromium (Hendler, 2001). Chromium nicotinate and chromium picolinate may be more bioavailable than chromium chloride (Kobla, 2000). In much of the research on impaired glucose tolerance and type-2 diabetes, chromium picolinate was the source of chromium. However, some concerns have been raised over the long-term

safety of chromium picolinate supplementation. There have been several studies involving chromium supplementation of subjects with non-insulin-dependent diabetes and/or lipemia that have reported beneficial effects of chromium on the glucose/insulin system (Mertz, 1992).

Chromium supplements given to people with impaired glucose tolerance or diabetes may lead to improved blood glucose, insulin and lipid variables. Chromium has also been shown to improve lean body mass in humans and swine. Studies on the effectiveness of chromium supplementation in patients with glucose intolerance and type II diabetes have produced conflicting results. Improvements have been noted in glucose tolerance tests in adult diabetics (Glinsmann and Mertz 1966, Mossop, 1983) and glucose intolerant men and women (Martinez 1985; Anderson 1983 and Anderson 1991).

Chromium supplementation may also have a favorable impact on triglycerides, LDL and HDL cholesterol (Abraham, 1991 and Press 1990). More recent human studies have shown favorable responses with chromium supplementation. Diets supplemented with chromium picolinate (200 ug/day) were compared to a placebo in 48 patients with type-1 diabetes and 114 patients with type-2 diabetes. In approximately 70% of patients supplemented with chromium, there was a reduced need for insulin or oral hypoglycemic medications (Ravina 1995).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

Because there was not enough information on chromium requirements to set a recommended dietary allowance (RDA), the Food and Nutrition Board set an adequate intake level (AI) based on the chromium content in normal diets (Food and Nutrition Board 2001).

Table 1.1
RECOMMENDED DIETARY ALLOWANCE (RDA) FOR CHROMIUM
(FOOD AND NUTRITION BOARD 2001)

Adequate Intake (AI) for Chromium			
Life Stage	Age	Males (mcg/day)	Females (mcg/day)
Infants	0-6 months	0.2	0.2
Infants	7-12 months	5.5	5.5
Children	1-3 years	11	11
Children	4-8 years	15	15
Children	9-13 years	25	21
Adolescents	14-18 years	35	24
Adults	19-50 years	35	25
Adults	51 years and older	30	20
Pregnancy	18 years and younger	-	29
Pregnancy	19 years and older	-	30
Breastfeeding	18 years and younger	-	44
Breastfeeding	19 years and older	-	45

TOXICITY

Most of the concerns regarding the long-term safety of chromium (III) supplementation arise from several studies in cell culture, suggesting chromium (III), especially in the form of chromium picolinate, may increase DNA damage (Stearns, 1995 and Blasiak, 2000). Presently there is no evidence that chromium (III) increases DNA damage in living organisms (Food and Nutrition Board 2001) and a study in 10 women taking 400 mcg/day of chromium as chromium picolinate found no evidence of increased oxidative damage to DNA as measured by antibodies to an oxidized DNA base (Kato, 1998).

Several studies have demonstrated the safety of daily doses of up to 1,000 mcg of chromium for several months (Anderson, 1997 and Hathcock, 1997). However, there

have been a few isolated reports of serious adverse reactions to chromium picolinate. Kidney failure was reported five months after a six-week course of 600 mcg of chromium/day in the form of chromium picolinate (Wasser, 1997). While kidney failure and impaired liver function were reported after the use of 1200-2400 mcg/day of chromium in the form of chromium picolinate over a period of four to five months (Cerulli, 1998). Individuals with pre-existing kidney or liver disease may be at increased risk of adverse effects and should limit supplemental chromium intake (Food and Nutrition Board 2001).

ZINC

The importance of zinc for normal growth and survival of plants and animals was recognized a long time ago. Yet the existence of its deficiency in humans was doubted because of the element's ubiquitous distribution in the environment and the lack of obvious clinical signs of deficiency. Nevertheless, evidence of human deficiency began to emerge during the 1960s, when cases of zinc-responsive dwarfism and delayed sexual maturation were first reported in Egyptian adolescents (Prasad, 1991). Since then, a number of intervention trials have been carried out to assess the impact of zinc supplementation, particularly in low-income populations who are likely to suffer from zinc deficiency (Brown, et al. 2002).

Zinc insufficiency has been recognized by a number of experts as an important public health issue, especially in developing countries (Prasad, 1998).

DIETARY SOURCES OF ZINC

Zinc occurs in a wide variety of foods, but is found in highest concentrations in animal sources, particularly beef, pork, poultry and fish, and in lesser amounts in eggs and dairy products. Zinc content is relatively high in nuts, legumes and whole grain cereals and is lower in fruits and vegetables.

Dietary factors can alter the proportion of zinc that is available for absorption in the intestine by as much as ten-fold. Most of the available information on the effect of specific dietary factors on zinc absorption has been derived from studies measuring

absorption from single test meals. Though these data may not reflect the true proportion of zinc absorbed from meals consumed over the whole day, they are useful to identify the factors that affect zinc absorption. The dietary components that have a substantial impact on the absorption of zinc are phytate and calcium, which inhibit zinc absorption, and protein, which enhances the absorption (Lonnardal, 2000).

Shellfish, beef, and other red meats are rich sources of zinc. Nuts and legumes are relatively good plant sources. Zinc bioavailability (the fraction of zinc retained and used by the body) is relatively high in meat, eggs, and seafood because of the relative absence of compounds that inhibit zinc absorption and the presence of certain amino acids (cysteine and methionine) that improve zinc absorption. The zinc in whole grain products and plant proteins is less bioavailable due to their relatively high content of phytic acid, a compound that inhibits zinc absorption (King, 1999).

FUNCTION

Zinc plays an important role in the structure of proteins and cell membranes. A finger-like structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (Cu Zn SOD), while zinc plays a critical structural role (Food and Nutrition Board 2001 & King, 1999). The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (O'Dell, 2000).

Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum. Specific transport proteins may facilitate the passage of zinc across the cell membrane into the portal circulation. With high intakes, zinc is also absorbed through a passive para cellular route.

The portal system carries absorbed zinc directly to the liver, and then released into systemic circulation for delivery to other tissues. About 70% of zinc in circulation is bound to albumin, and any condition that alters serum albumin concentration can have a

secondary effect on serum zinc levels. Although serum zinc represents only 0.1% of the whole body zinc, the circulating zinc turns over rapidly to meet tissue needs.

Loss of zinc through gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. Considerable amount of zinc is secreted through the biliary and intestinal secretions, but most of it is reabsorbed and this process is an important point of regulation of zinc balance. Other routes of zinc excretion include the urine and surface losses (desquamated skin, hair, sweat). Zinc finger proteins have been found to regulate gene expression by acting as transcription factors (binding to DNA and influencing the transcription of specific genes). Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission. Recently zinc has been found to play a role in apoptosis (gene-directed cell death), a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Truong-Tran, 2000).

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function. If zinc is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc malabsorption resulting in severe deficiency, have provided much insight into the functional outcomes of zinc deficiency (Van Wouwe, 1989)

NUTRIENT INTERACTIONS

Copper: Taking large quantities of zinc (50 mg/day or more) over a period of weeks can interfere with copper bioavailability. High intake of zinc induces the intestinal synthesis of a copper-binding protein called metallothionein. Metallothionein traps copper within intestinal cells and prevent its systemic absorption. More typical intakes of zinc do not affect copper absorption and high copper intakes do not affect zinc absorption (King, 1999).

Iron: Supplemental (38-65 mg/day of elemental iron) but not dietary levels of iron may decrease zinc absorption. This interaction is of concern in the management of iron supplementation during pregnancy and lactation and has led some experts to recommend zinc supplementation for pregnant and lactating women taking more than 60 mg/day of elemental iron (O'Brien, 2000 and Fung, 1997).

Calcium: High levels of dietary calcium impair zinc absorption in animals, but it is uncertain whether this occurs in humans. Increasing the calcium intake of postmenopausal women by 890 mg/day in the form of milk or calcium phosphate (total calcium intake 1,360 mg/day) reduced zinc absorption and zinc balance in postmenopausal women (Wood, 1997), but increasing the calcium intake of adolescent girls by 1,000 mg/day in the form of calcium citrate malate (total calcium intake 1,667 mg/day) did not affect zinc absorption or balance (McKenna, 1997). Calcium in combination with phytic acid reduces zinc absorption. This effect is particularly relevant to individuals consuming a diet that is highly dependent on tortillas made with lime (calcium oxide).

Folic acid: The bioavailability of dietary folate is increased by the action of a zinc-dependent enzyme, suggesting a possible interaction between zinc and folic acid. In the past, some studies found low zinc intake to decrease folate absorption, while other studies found folic acid supplementation to impair zinc utilization in individuals with marginal zinc status (Food and Nutrition Board 2001 & King, 1999). However, a more recent study found that supplementation with a relatively high dose of folic acid (800 mcg/day) for 25 days did not alter zinc status in a group of students being fed low-zinc diets (3.5 mg/day), nor did zinc intake impair folate utilization (Kauwell, 1995).

DEFICIENCY

Severe Zinc deficiency

Much of what is known about severe zinc deficiency was derived from the study of individuals born with acrodermatitis enteropathica, a genetic disorder resulting from the impaired uptake and transport of zinc. The symptoms of severe zinc deficiency

include the slowing or cessation of growth and development, delayed sexual maturation, characteristic skin rashes, chronic and severe diarrhea, immune system deficiencies, impaired wound healing, diminished appetite, impaired taste sensation, night blindness, swelling and clouding of the corneas, and behavioral disturbances. Before the cause of acrodermatitis enteropathica was known, patients typically died in infancy. Oral zinc therapy results in the complete remission of symptoms, though it must be maintained indefinitely in individuals with the genetic disorder (King, 1999 and Hambidge, 2000). Although dietary zinc deficiency is unlikely to cause severe zinc deficiency in individuals without a genetic disorder, zinc malabsorption or conditions of increased zinc loss, such as severe burns or prolonged diarrhea, may also result in severe zinc deficiency.

Mild zinc deficiency

More recently, it has become apparent that milder zinc deficiency contributes to a number of health problems, especially common in children who live in developing countries. The lack of a sensitive indicator of mild zinc deficiency hinders the scientific study of its health implications. However, controlled trials of moderate zinc supplementation have demonstrated that mild zinc deficiency contributes to impaired physical and neuropsychological development, and increased susceptibility to life-threatening infections in young children (Hambidge, 2000).

Individuals at risk of zinc deficiency: (King, 1999)

- ✓ Infants and children
- ✓ Pregnant and lactating (breastfeeding) women, especially teenagers
- ✓ Patients receiving total parenteral nutrition (intravenous feedings)
- ✓ Malnourished individuals, including those with protein-energy malnutrition and anorexia nervosa
- ✓ Individuals with severe or persistent diarrhea
- Individuals with malabsorption syndromes, including sprue and short bowel syndrome
- ✓ Individuals with alcoholic liver disease have increased urinary zinc excretion and low liver zinc levels

- ✓ Individuals with sickle cell anemia
- ✓ Older adults (65 years and older)

Strict vegetarians: The requirement for dietary zinc may be as much as fifty percent greater for strict vegetarians whose major food staples are grains and legumes because high levels of phytic acid in these foods reduce the absorption of zinc (Food and Nutrition Board 2001).

DISEASE TREATMENT AND PREVENTION

Diabetes Mellitus:

Moderate zinc deficiency may be relatively common in individuals with diabetes mellitus. Increased urinary zinc excretion appears to contribute to the marginal zinc nutritional status that has been observed in diabetics (Blostein, 1997). Although zinc supplementation has been reported to improve immune function in diabetics and zinc supplementation of 50 mg/day adversely affected control of blood glucose in insulin-dependent (type-1) diabetics (Cunningham, 1994).

The influence of zinc on glucose metabolism requires further study before high-dose zinc supplementation can be advocated for diabetics (King, 1999).

Plasma zinc concentrations were reduced and urinary zinc excretion was elevated in diabetic patients (Kinlaw, et al. 1983; Tarui, 1963 ;Meltzer , et al. 1962 and Kumar , 1974).

Patients with zinc deficiency resulting from gastrointestinal diseases had significantly higher glucose levels and significantly lower insulin levels than did similar patients without zinc deficiency (Wolman et al, 1979).

Zinc supplementation increased plasma insulin levels in these patients. Healthy male volunteers consuming a low-zinc diet had a significant increase in fasting blood glucose levels (Solomon, 1982).

Impaired glucose tolerance also developed in rats fed a zinc deficient diet (Hendricks, 1972).

OTHER DISEASES

Age-related macular degeneration

A leading cause of blindness in people over the age of 65 in the U.S. is a degenerative disease of the macula, known as age-related macular degeneration (AMD). In the back of the eye, the macula is the portion of the retina involved with central vision. Zinc is hypothesized to play a role in the development of AMD for several reasons: (1) zinc is found in high concentrations in the part of the retina affected by AMD (2) retinal zinc content has been shown to decline with age, and (3) the activity of some zinc-dependent retinal enzymes has been shown to decline with age. However, scientific evidence that zinc intake is associated with the development or progression of AMD is limited. Observational studies have not demonstrated clear associations between dietary zinc intake and the incidence of AMD (Vanden Langenberg, 1998 and Cho, 2001). A randomized controlled trial provoked interest when it found that 200 mg/day of zinc sulfate (81 mg/day of elemental zinc) over 2 years reduced the loss of vision in patients with AMD (Newsome, 1988). However, a later trial using the same dose and duration found no beneficial effect in patients with a more advanced form of AMD in one eye (Stur, 1996). At present, there is little evidence that zinc supplementation would be beneficial to people with early signs of macular degeneration, but further randomized controlled trials are warranted (Evans, 2002).

HIV/AIDS

Sufficient zinc is essential in maintaining immune system function and HIV infected individuals are particularly susceptible to zinc deficiency. Decreased serum zinc levels have been associated with more advanced disease and increased mortality in HIV patients (Lai, 2001 and Wellinghausen, 2000).

In one of the few zinc supplementation studies conducted in AIDS patients, 45 mg/day of zinc for one month resulted in a decreased incidence in opportunistic infections compared to placebo (Mocchegiani, 2000). However, the HIV virus also requires zinc, and excessive zinc intake may stimulate the progression of HIV infection. In an

observational study of HIV-infected men, increased zinc intake was associated with more rapid disease progression and any intake of zinc supplements was associated with poorer survival (Tang, 1996). These results indicate that further research is necessary to determine optimal zinc intakes for HIV-infected individuals (Baum, 2000).

Significant delays in linear growth and weight gain, known as growth retardation or failure to thrive, are common features of mild zinc deficiency in children. In the 1970s and 1980s, several randomized placebo-controlled studies of zinc supplementation in young children with significant growth delays were conducted in Denver, Colorado. Modest zinc supplementation (5.7 mg/day) resulted in increased growth rates compared to placebo (Walravens, 1989).

More recently, a number of larger studies in developing countries observed similar results with modest zinc supplementation. A meta-analysis of growth data from zinc intervention trials recently confirmed the widespread occurrence of growth limiting zinc deficiency in young children, especially in developing countries (Hambidge, 2000).

Although the exact mechanism for the growth limiting effects of zinc deficiency are not known, recent research indicates that zinc availability affects cell signaling systems that coordinate the response to the growth-regulating hormone, insulin-like growth factor-1 (IGF-1) (MacDonald, 2000).

SUPPLEMENTS

Low maternal zinc nutritional status has been associated with diminished attention in the newborn infant and poorer motor function at 6 months of age. Zinc supplementation has been associated with improved motor development in very low-birth-weight infants, more vigorous activity in Indian infants and toddlers, and more functional activity in Guatemalan infants and toddlers (Caulfield, 1998). Additionally, zinc supplementation was associated with better neuropsychologic functioning (e.g., attention) in Chinese first grade students, but only when zinc was provided with other micronutrients (Sandstead, 1998). Two other studies failed to find an association between zinc supplementation and measures of attention in children diagnosed with growth retardation. Although initial studies suggest that zinc deficiency may depress cognitive

development in young children, more controlled research is required to determine the nature of the effect and whether zinc supplementation is beneficial (Black, 1998).

Adequate zinc intake is essential in maintaining the integrity of the immune system (Baum, 2000) and zinc deficient individuals are known to experience increased susceptibility to a variety of infectious agents (Shankar, 1998).

It is estimated that diarrheal diseases result in the deaths of over 3 million children in developing countries each year. The adverse effects of zinc deficiency on immune system function are likely to increase the susceptibility of children to infectious diarrhea, while persistent diarrhea contributes to zinc deficiency and malnutrition. Recent research indicates that zinc deficiency may also potentiate the effects of toxins produced by diarrhea-causing bacteria like *E. coli* (Wapnir, 2000). Zinc supplementation in combination with oral rehydration therapy has been shown to significantly reduce the duration and severity of acute and persistent childhood diarrhea and to increase survival in a number of randomized controlled trials (Bhutta, 2000).

Zinc supplementation may also reduce the incidence of lower respiratory infections, such as pneumonia. A pooled analysis of a number of studies in developing countries demonstrated a substantial reduction in the prevalence of pneumonia in children supplemented with zinc (Bhutta, 1999).

Several studies have indicated that zinc supplementation may reduce the incidence of clinical attacks of malaria in children (Black, 1998). A placebo-controlled trial in preschool children in Papua New Guinea found that zinc supplementation reduced the frequency of health center attendance due to *plasmodium falciparum* malaria by 38% (Shankar, 2000). Additionally, the number of malaria episodes accompanied by high blood levels of the malaria-causing parasite was reduced by 68%, suggesting that zinc supplementation may be of benefit in preventing more severe episodes of malaria. However, a 6-month trial in more than 700 West African children did not find the frequency or severity of malaria episodes caused by *P. falciparum* to be different in children supplemented with zinc compared to those given a placebo (Muller, 2001).

Age-related declines in immune function are similar to those associated with zinc deficiency, and the elderly represent a group that is vulnerable to mild zinc deficiency. However, the results of zinc supplementation trials on immune function in the elderly have been mixed. Certain aspects of immune function in the elderly have been found to improve with zinc supplementation (Salgueiro, 2000). In a randomized placebo-controlled study in men and women over 65 years of age was found that a zinc supplement of 25 mg/day for 3 months increased levels of some circulating immune cells (CD4 T-cells and cytotoxic T-lymphocytes) compared to placebo (Fortes, 1998).

However, other studies have not found zinc supplementation to improve parameters of immune function, indicating that more research is required before any recommendations regarding zinc and immune system response in the elderly can be made.

It has been estimated that 82% of pregnant women worldwide are likely to have inadequate zinc intakes. Poor maternal zinc nutritional status has been associated with a number of adverse outcomes of pregnancy, including low birth weight, premature delivery, and labor and delivery complications. However, the results of maternal zinc supplementation trials in the U.S. and developing countries have been mixed (Caulfield, 1998). Although some studies have found maternal zinc supplementation to increase birth weight and decrease the likelihood of premature delivery, two recent studies in Peruvian and Bangladeshi women found no difference between zinc supplementation and placebo in the incidence of low birth weight or premature delivery (Caulfield, 1999 and Osendarp, 2000). Supplementation studies designed to examine the effect of zinc supplementation on labor and delivery complications have also generated mixed results, though few have been conducted in zinc deficient populations (Caulfield, 1998).

A number of zinc supplements are available, including zinc acetate, zinc gluconate, zinc picolinate and zinc sulfate. Zinc picolinate has been promoted as a more absorbable form of zinc, but there is little data to support this idea in humans. Limited work in animals suggests that increased intestinal absorption of zinc picolinate may be offset by increased elimination (Food and Nutrition Board 2001).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

The U.S. recommended dietary allowances (RDA) for zinc are listed for all age groups because infants, children, and pregnant and lactating women are at increased risk of zinc deficiency. Since a sensitive indicator of zinc nutritional status is not readily available, the RDA for zinc was based on a number of different indicators of zinc nutritional status and represents the daily intake likely to prevent deficiency in nearly all individuals in a specific age and gender group (Food and Nutrition Board 2001).

Table 1.2
RECOMMENDED DIETARY ALLOWANCE (RDA) FOR ZINC
(FOOD AND NUTRITION BOARD 2001)

The Recommended Dietary Allowance (RDA) for Zinc			
Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	2	2
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger	-	12
Pregnancy	19 years and older	-	11
Breastfeeding	18 years and younger	-	13
Breastfeeding	19 years and older	-	12

TOXICITY

Acute toxicity

Isolated outbreaks of acute zinc toxicity have occurred as a result of the consumption of food or beverages contaminated with zinc released from galvanized containers. Signs of acute zinc toxicity are abdominal pain, diarrhea, nausea, and vomiting. Single doses of 225 to 450 mg of zinc usually induce vomiting. Milder gastrointestinal distress has been reported at doses of 50 to 150 mg/day of supplemental

zinc. Metal fume fever has been reported after the inhalation of zinc oxide fumes. Profuse sweating, weakness, and rapid breathing may develop within 8 hours of zinc oxide inhalation and persist 12-24 hours after exposure is terminated (Food and Nutrition Board 2001 & King, 1999)

Adverse effects

The major consequence of long-term consumption of excessive zinc is copper deficiency. Total zinc intakes of 60 mg/day (50 mg supplemental and 10 mg dietary zinc) have been found to result in signs of copper deficiency. In order to prevent copper deficiency, the U.S. Food and Nutrition Board set the tolerable upper level (UL) of intake for adults at 40 mg/day, including dietary and supplemental zinc (Food and Nutrition Board 2001).

Table 1.3
TOLERABLE UPPER INTAKE LEVEL (UL) FOR ZINC
(FOOD AND NUTRITION BOARD 2001)

Tolerable Upper Intake Level (UL) for Zinc	
Age Group	UL (mg/day)
Infants 0-6 months	4
Infants 7-12 months	5
Children 1-3 years	7
Children 4-8 years	12
Children 9-13 years	23
Adolescents 14-18 years	34
Adults 19 years and older	40

MANGANESE

Manganese is a mineral element that is both nutritionally essential and potentially toxic. The derivation of its name from the Greek word for magic remains appropriate because scientists are still working to understand the diverse effects of manganese deficiency and manganese toxicity in living organisms (Keen, 1999).

DIETARY SOURCES OF MANGANESE:

Estimated average dietary manganese intakes in the U.S. range from 2.1-2.3 mg/day for men and 1.6-1.8 mg/day for women. People eating vegetarian diets and western diets emphasizing whole grains may have manganese intakes as high as 10.9 mg/day (Food and Nutrition Board 2001).

Rich sources of manganese include whole grains, nuts, leafy vegetables, and teas. Foods high in phytic acid, such as beans, seeds, nuts, whole grains, and soy products, or foods high in oxalic acid, such as cabbage, spinach, and sweet potatoes, may slightly inhibit manganese absorption. Although teas are rich sources of manganese, the tannins present in tea may moderately reduce the absorption of manganese (Kies, 1994).

FUNCTION

Manganese (Mn) plays an important role in a number of physiologic processes as a constituent of some enzymes and an activator of other enzymes (Nielsen, 1999).

Antioxidant function

Manganese superoxide dismutase (MnSOD) is the principal antioxidant enzyme of mitochondria. Because mitochondria consume over 90% of the oxygen used by cells, they are especially vulnerable to oxidative stress. The superoxide radical is one of the reactive oxygen species produced in mitochondria during ATP synthesis. MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which can be reduced to water by other antioxidant enzymes (Leach, 1997).

Metabolism

A number of manganese-activated enzymes play important role in the metabolism of carbohydrates, amino acids, and cholesterol (Food and Nutrition Board 2001). Pyruvate carboxylase, a manganese-containing enzyme, and phosphoenolpyruvate carboxy kinase (PEPCK), a manganese-activated enzyme, play critical roles in

gluconeogenesis—the production of glucose from non-carbohydrate precursors. Arginase, another manganese-containing enzyme, is required by the liver for the urea cycle, a process that detoxifies ammonia generated during amino acid metabolism (Leach, 1997).

NUTRIENT INTERACTIONS

Iron: Although the specific mechanisms for manganese absorption and transport have not been determined, some evidence suggests that iron and manganese can share common absorption and transport pathways. Absorption of manganese from a meal is reduced as the meal's iron content is increased (Keen, 1996).

Iron supplementation (60 mg/day for 4 months) was associated with decreased blood manganese levels and decreased MnSOD activity in white blood cells, indicating a reduction in manganese nutritional status (Davis, 1992).

An individual's iron status can affect manganese bioavailability. Intestinal absorption of manganese is increased during iron deficiency, and increased iron stores (ferritin levels) are associated with decreased manganese absorption (Finley, 1999). The finding that men generally absorb less manganese than women may be related to the fact that men usually have higher iron stores than women (Finley, 1994).

Magnesium: Supplemental magnesium (200mg/day) decreased manganese bioavailability slightly, either by decreasing manganese absorption or by increasing its loss in healthy adults (Kies, 1994).

Calcium: In one set of studies, supplemental calcium (500 mg/day) resulted in slightly lower manganese bioavailability in healthy adults. As a source of calcium, milk had the least effect, while calcium carbonate and calcium phosphate had the greatest effect (Kies, 1994). Several others studies have found the effect of supplemental calcium on manganese metabolism to be minimal (Johnson, 1991).

DEFICIENCY

Manganese deficiency has been observed in a number of animal species. Signs of manganese deficiency include impaired growth, impaired reproductive function, skeletal abnormalities, impaired glucose tolerance, and altered carbohydrate and lipid metabolism. In humans, demonstration of a manganese deficiency syndrome has been less clear (Nielsen, 1999 and Keen, 1996).

A child on long-term total parenteral nutrition (TPN) that lacked manganese developed bone demineralization and impaired growth that were corrected by manganese supplementation (Norose, 1992). Young men who were fed a low-manganese diet developed decreased serum cholesterol levels and a transient skin rash (Friedman, 1987).

Blood calcium, phosphorus, and alkaline phosphatase levels were also elevated, which may indicate increased bone remodeling as a consequence of insufficient dietary manganese. Young women fed a manganese-poor diet developed mildly abnormal glucose tolerance in response to an intravenous (IV) infusion of glucose (Johnson, 1991).

DISEASE TREATMENT AND PREVENTION

Diabetes mellitus

Manganese deficiency results in glucose intolerance similar to diabetes mellitus in some animal species, but studies examining the manganese status of diabetic humans have generated mixed results. Whole blood manganese levels did not differ significantly between 57 diabetics and 28 non-diabetic controls (Walter, 1991). However, urinary manganese excretion tended to be slightly higher in 185 diabetics compared to 185 non-diabetic controls (el-Yazigi 1991). Additionally, a study of functional manganese status found the activity of the antioxidant enzyme, MnSOD, to be lower in the white blood cells of diabetics than in those of non-diabetic controls (Nath, 1984).

OTHER DISEASES

Bone development

Manganese deficiency results in abnormal skeletal development in a number of animal species. Manganese is the preferred cofactor of enzymes called glycosyltransferases, which are required for the synthesis of proteoglycans that are needed for the formation of healthy cartilage and bone (Keen, 1996).

Wound healing

Wound healing is a complex process that requires increased production of collagen. Manganese is required for the activation of prolydase, an enzyme that functions to provide the amino acid, proline for collagen formation in human skin cells (Muszynska, 2000).

A genetic disorder known as prolydase deficiency results in abnormal wound healing among other problems, and is characterized by abnormal manganese metabolism (Keen, 1996). Glycosaminoglycan synthesis, which requires manganese-activated glycosyltransferases, may also play an important role in wound healing (Shetlar, 1994).

Epilepsy (seizure disorders)

Manganese deficient rats are more susceptible to seizures, and rats that are genetically prone to epilepsy have lower than normal brain and blood manganese levels. Certain subgroups of humans with epilepsy have been found to have lower whole blood manganese levels than non-epileptic controls. One study found blood manganese levels of individuals with epilepsy of unknown origin to be lower than those of individuals whose epilepsy was induced by trauma (e.g., head injury) or disease, suggesting a possible genetic relationship between epilepsy and abnormal manganese metabolism. While manganese deficiency does not appear to be a cause of epilepsy in humans, the relationship between manganese metabolism and epilepsy deserves further research (Keen, 1996 and Carl, 1994).

SUPPLEMENTS

Several forms of manganese are found in supplements, including manganese gluconate, manganese sulfate, manganese ascorbate, and amino acid chelates of manganese. Manganese is available as a stand-alone supplement or in combination products (Hendler, 2001).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

Because there was not enough information on manganese requirements to set a Recommended Dietary Allowance (RDA), the Food and Nutrition Board (FNB) of the Institute of Medicine set an adequate intake level (AI). Since overt manganese deficiency has not been documented in humans eating natural diets, the FNB based the AI on average dietary intakes of manganese determined by the Total Diet Study — an annual survey of the mineral content of representative diets of Americans (Food and Nutrition Board 2001). Adequate Intake values for manganese are listed in the table below in milligrams/day by age and gender.

Table 1.4
RECOMMENDED DIETARY ALLOWANCE (RDA)
FOOD AND NUTRITION BOARD FOR MANGANESE 2001

Adequate Intake (AI) for Manganese			
Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	0.003	0.003
Infants	7-12 months	0.6	0.6
Children	1-3 years	1.2	1.2
Children	4-8 years	1.5	1.5
Children	9-13 years	1.9	1.6
Adolescents	14-18 years	2.2	1.6
Adults	19 years and older	2.3	1.8
Pregnancy	all ages	-	2.0
Breastfeeding	all ages	-	2.6

TOXICITY

Inhaled manganese: Manganese toxicity may result in multiple neurologic problems and is a well-recognized health hazard for people who inhale manganese dust (Keen, 1999 & Food and Nutrition Board 2001). Unlike ingested manganese, inhaled manganese is transported directly to the brain before it can be metabolized in the liver (Davis, 1998).

The symptoms of manganese toxicity generally appear slowly over a period of months to years. In its worst form, manganese toxicity can result in a permanent neurological disorder with symptoms similar to those of Parkinson's disease, including tremors, difficulty walking, and facial muscle spasms. This syndrome is sometimes preceded by psychiatric symptoms, such as irritability, aggressiveness and even hallucinations (Pal, 1999).

Ingested manganese: Water manganese levels were found to be 14 mg/liter almost 2 months after symptoms began and may have already been declining (Keen, 1999). A study of older adults in Greece found a high prevalence of neurological symptoms in those exposed to water manganese levels of 1.8-2.3 mg/liter (Kondakis, 1989). While a study of people in Germany, drinking water with manganese levels ranging from 0.3-2.2 mg/liter found no evidence of increased neurological symptoms compared to those drinking water containing less than 0.05 mg/liter (Vieregge, 1995).

A single case of manganese toxicity was reported in a person who took large amounts of mineral supplements for years (Keen, 1994), while another case was reported as a result of taking a Chinese herbal supplement (Pal, 1999).

Individuals with increased susceptibility to manganese toxicity

Chronic liver disease: Manganese is eliminated from the body mainly in bile. Thus, impaired liver function may lead to decreased manganese excretion. Manganese accumulation in individuals with cirrhosis or liver failure may contribute to neurological problems and Parkinson's disease-like symptoms (Keen, 1999 and Hendler, 2001).

Newborns: The newborn brain may be more susceptible to manganese toxicity due to a greater expression of receptors for the manganese transport protein (transferrin) in developing nerve cells and the immaturity of the liver's bile elimination system (Food and Nutrition Board 2001).

Due to the severe implications of manganese neurotoxicity the Food and Nutrition Board (FNB) of the Institute of Medicine set very conservative upper levels of intake (UL) for manganese, which are listed in the table below (Food and Nutrition Board 2001).

Table 1.5
TOLERABLE UPPER INTAKE LEVEL (UL) FOR MANGANESE
(FOOD AND NUTRITION BOARD 2001)

Tolerable Upper Intake Level (UL) for Manganese	
Age Group	UL (mg/day)
Infants 0-12 months	Not possible to establish*
Children 1-3 years	2
Children 4-8 years	3
Children 9-13 years	6
Adolescents 14-18 years	9
Adults 19 years and older	11

*Source of intake should be from food and formula only.

COPPER

Copper (Cu) is an essential trace element for humans and animals. In the body, copper shifts between the cuprous (Cu^{1+}) and the cupric (Cu^{2+}) forms, though the majority of the body's copper is in the Cu^{2+} form. The ability of copper to easily accept and donate electrons explain its important role in oxidation-reduction (redox) reactions and the scavenging of free radicals (Linder, 1996).

DIETARY SOURCES OF COPPER

Copper is found in a wide variety of foods and is most plentiful in organ meats, shellfish, nuts, and seeds. Wheat bran cereals and whole grain products are also good sources of copper. According to national surveys, the average dietary intake of copper in

the U.S. is approximately 1.0 to 1.1 mg (1000 to 1100 mcg)/day for adult women and 1.2 to 1.6 mg (1200 to 1600 mcg)/day for adult men (Food and Nutrition Board 2001).

FUNCTION

Copper is a critical functional component of a number of essential enzymes, known as cupro enzymes. The copper-dependent enzyme, cytochrome *c* oxidase, plays a critical role in cellular energy production. By catalyzing the reduction of molecular oxygen (O_2) to water (H_2O), cytochrome *c* oxidase generates an electrical gradient used by the mitochondria to create the vital energy-storing molecule, ATP (Uauy, 1998).

Connective tissue formation

Another cuproenzyme, lysyl oxidase, is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue. The action of lysyl oxidase helps maintain the integrity of connective tissue in the heart and blood vessels and plays a role in bone formation (Turnlund, 1999).

Iron metabolism

Two copper-containing enzymes, ceruloplasmin (ferroxidase I) and ferroxidase II have the capacity to oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation. Although the ferroxidase activity of these two cuproenzymes has not yet been proven to be physiologically significant, the fact that iron mobilization from storage sites is impaired in copper deficiency supports their role in iron metabolism (Turnlund, 1999 and Harris, 1997).

Central nervous system: A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes.

Neurotransmitter synthesis: Dopamine- β -monooxygenase catalyzes the conversion of dopamine to the neurotransmitter nor epinephrine (Harris, 1997).

Metabolism of neurotransmitters: Monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine and dopamine. MAO also functions in the degradation of the neurotransmitter serotonin, which is the basis for the use of MAO inhibitors as antidepressants (Food and Nutrition Board 2001).

Formation and maintenance of myelin: The myelin sheath is made of phospholipids whose synthesis depends on cytochrome *c* oxidase activity.

Melanin formation

The cuproenzyme, tyrosinase, is required for the formation of the pigment melanin. Melanin is formed in cells called melanocytes and plays a role in the pigmentation of the hair, skin, and eyes (Turnlund, 1999).

Antioxidant functions

Superoxide dismutase: Superoxide dismutase (SOD) functions as an antioxidant by catalyzing the conversion of superoxide radicals (free radicals) to hydrogen peroxide, which can subsequently be reduced to water by other antioxidant enzymes (Johnson, 1992). Two forms of SOD contain copper: (1) copper/zinc SOD is found within most cells of the body, including red blood cells, and (2) extra cellular SOD is a copper containing enzyme found in high levels in the lungs and low levels in blood plasma (Turnlund, 1999).

Ceruloplasmin: Ceruloplasmin may function as an antioxidant in two different ways. Free copper and iron ions are powerful catalysts of free radical damage. By binding copper, ceruloplasmin prevents free copper ions from catalyzing oxidative damage. The ferroxidase activity of ceruloplasmin (oxidation of ferrous iron) facilitates iron loading onto its transport protein, transferrin, and may prevent free ferrous ions (Fe^{2+}) from participating in harmful free radical generating reactions (Johnson, 1992).

Regulation of gene expression

Copper-dependent transcription factors regulate transcription of specific genes. Thus, cellular copper levels may affect the synthesis of proteins by enhancing or

inhibiting the transcription of specific genes. Genes regulated by copper-dependent transcription factors include genes for copper/zinc superoxide dismutase (Cu/Zn, SOD), catalase (another antioxidant enzyme), and proteins related to the cellular storage of copper (Uauy, 1998).

NUTRIENT-NUTRIENT INTERACTIONS

Iron: Adequate copper nutritional status appears to be necessary for normal iron metabolism and red blood cell formation. Anemia is a clinical sign of copper deficiency, and iron has been found to accumulate in the livers of copper deficient animals, indicating that copper (probably in the form of ceruloplasmin) is required for iron transport to the bone marrow for red blood cell formation. Infants fed a high iron formula absorbed less copper than infants fed a low iron formula, suggesting that high iron intakes may interfere with copper absorption in infants.

Zinc: High supplemental zinc intakes of 50 mg/day or more for extended periods of time may result in copper deficiency. High dietary zinc increases the synthesis of an intestinal cell protein called metallothionein, which binds certain metals and prevents their absorption by trapping them in intestinal cells. Metallothionein has a stronger affinity for copper than zinc, so high levels of metallothionein induced by excess zinc cause a decrease in intestinal copper absorption. High copper intakes have not been found to affect zinc nutritional status.

Fructose: High fructose diets have exacerbated copper deficiency in rats, but not in pigs whose gastrointestinal systems are more like those of humans. Very high levels of dietary fructose (20% of total calories) did not result in copper depletion in humans, suggesting that fructose intake does not result in copper depletion at levels relevant to normal diets (Turnlund, 1999 & Food and Nutrition Board 2001).

Vitamin C: Although vitamin C supplements have produced copper deficiency in laboratory animals, the effect of vitamin C supplements on copper nutritional status in humans is less clear. Two small studies in healthy young adult men indicate that the oxidase activity of ceruloplasmin may be impaired by relatively high doses of supplemental vitamin C. In one study, vitamin C supplementation of 1500 mg/day for 2

months resulted in a significant decline in ceruloplasmin oxidase activity (Finley, 1983). In the other study, supplements of 605 mg of vitamin C/day for 3 weeks resulted in decreased ceruloplasmin oxidase activity, although copper absorption did not decline (Jacob, 1987). Neither of these studies found vitamin C supplementation to adversely affect copper nutritional status.

DEFICIENCY

Individuals at risk of deficiency

Cow's milk is relatively low in copper, and cases of copper deficiency have been reported in high-risk infants and children fed only cow's milk formula. High-risk individuals include: premature infants, especially those with low-birth weight, infants with prolonged diarrhea, infants and children recovering from malnutrition, individuals with malabsorption syndromes, including celiac disease, sprue, and short bowel syndrome due to surgical removal of a large portion of the intestine. Individuals receiving intravenous total parenteral nutrition or other restricted diets may also require supplementation with copper and other trace elements (Uauy, 1998 and Turnlund, 1999). Recent research indicates that cystic fibrosis patients may also be at increased risk of copper insufficiency (Percival, 1999).

DISEASE TREATMENT AND PREVENTION

Diabetes Mellitus

Copper deficiency in experimental animals resulted in impaired glucose tolerance (Hassel, et al. 1983) and increased concentrations of glycosylated hemoglobin (indicative of chronic hyperglycemia) (Klevay, 1982). Because the typical American diet contains only about half of the Recommended Dietary Allowance (2 mg/day) of copper (Wolf, et al. 1977) a deficiency of this mineral may be common.

OTHER DISEASES

Cardiovascular diseases

While it is clear that severe copper deficiency results in heart abnormalities and damage (cardiomyopathy) in some animal species, the pathology differs from

atherosclerotic cardiovascular diseases prevalent in humans (Food and Nutrition Board 2001). Studies in humans have produced inconsistent results, and their interpretation is hindered by the lack of a reliable marker of copper nutritional status. Outside the body, free copper is known to be a pro-oxidant and is frequently used to produce oxidation of low density lipoprotein (LDL) in the test tube. Recently, the copper-containing protein ceruloplasmin has been found to stimulate LDL oxidation in the test tube (Fox, 2000), leading some scientists to propose that increased copper levels could increase the risk of atherosclerosis by promoting the oxidation of LDL. However, there is little evidence that copper or ceruloplasmin promotes LDL oxidation in the human body. Additionally, the cuproenzymes, superoxide dismutase and ceruloplasmin, are known to have antioxidant properties, leading some experts to propose that copper deficiency rather than excess copper increases the risk of cardiovascular disease. Several epidemiological studies have found increased serum copper levels to be associated with increased risk of cardiovascular disease. While studies in very small numbers of adults fed experimental diets low in copper have demonstrated adverse changes in blood cholesterol levels, including increased total and LDL-cholesterol levels and decreased HDL-cholesterol levels (Klevay, 1998), other studies have not confirmed those results (Milne, 1996).

Copper supplementation of 2-3 mg/day for 4 to 6 weeks did not result in clinically significant changes in cholesterol levels (Medeiros, 1991 and Jones, 1997).

Recent research has also failed to find evidence that increased copper intake increases oxidative stress. In a multi-center placebo-controlled study, copper supplementation of 3 and 6 mg/day for 6 weeks did not result in increased susceptibility of LDL to oxidation induced outside the body (*in vitro*) by copper or peroxynitrite (a reactive nitrogen species) (Turley, 2000). Moreover, supplementation with 3 and 6 mg/day of copper decreased the *in vitro* oxidizability of red blood cells (Rock, 2000), indicating that relatively high intakes of copper do not increase the susceptibility of LDL or red blood cells to oxidation.

Immune system function

Copper is known to play an important role in the development and maintenance of immune system function, but the exact mechanism of its action is not yet known.

Neutropenia (abnormally low numbers of white blood cells called neutrophils) is a clinical sign of copper deficiency in humans. Adverse effects of insufficient copper on immune function appear most pronounced in infants. Infants with Menkes disease, a genetic disorder that results in severe copper deficiency, suffer from frequent and severe infections (Percival, 1998 and Failla, 1998). In a study of 11 malnourished infants with evidence of copper deficiency, the ability of certain white blood cells to engulf pathogens increased significantly after one month of copper supplementation (Heresi, 1985). More recently, 11 men on a low-copper diet (0.66 mg copper/day for 24 days and 0.38 mg/day for another 40 days) showed a decreased proliferation response when white blood cells called mononuclear cells isolated from their blood were presented with an immune challenge in cell culture (Kelley, 1995). While severe copper deficiency has adverse effects on immune function, the effects of marginal copper insufficiency in humans are not yet clear.

Osteoporosis

The copper-dependent enzyme, lysyl oxidase, is required for the maturation (cross-linking) of collagen, a key element in the organic matrix of bone. Osteoporosis has been observed in infants and adults with severe copper deficiency, but it is not clear whether marginal copper deficiency contributes to osteoporosis. Research regarding the role of copper nutritional status in age-related osteoporosis is limited. Serum copper levels of 46 elderly patients with hip fractures were reported to be significantly lower than matched control (Conlan, 1990). A small study in perimenopausal women, who consumed an average of 1 mg of dietary copper daily, reported decreased loss of bone mineral density (BMD) from the lumbar spine after copper supplementation of 3 mg/day for 2 years (Eaton, 1996). Marginal copper intake of 0.7 mg/day for 6 weeks significantly increased a measurement of bone resorption (breakdown) in healthy adult males (Baker, 1999). However, copper supplementation of 3 to 6 mg/day for 6 weeks had no effect on biochemical markers of bone resorption or bone formation in a study of healthy adult men and women (Baker, et al, 1999). Although severe copper deficiency is known to adversely affect bone health, the effects of marginal copper deficiency and copper supplementation on bone metabolism and age-related osteoporosis require further research before conclusions can be drawn.

SUPPLEMENTS

Copper supplements are available as cupric oxide, copper gluconate, copper sulfate, and copper amino acid chelates (Hendler, 2001).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

A variety of indicators were used to establish the recommended dietary allowance (RDA) for copper, including plasma copper concentration, serum ceruloplasmin activity, superoxide dismutase activity in red blood cells, and platelet copper concentration (Food and Nutrition Board, 2001). The RDA for copper reflects the results of depletion-repletion studies and is based on the prevention of deficiency.

Table 1.6
RECOMMENDED DIETARY ALLOWANCE (RDA) FOR COPPER
(FOOD AND NUTRITION BOARD 2001)

Recommended Dietary Allowance (RDA) for Copper			
Life Stage	Age	Males (mcg/day)	Females (mcg/day)
Infants	0-6 months	200 (AI)	200 (AI)
Infants	7-12 months	220 (AI)	220 (AI)
Children	1-3 years	340	340
Children	4-8 years	440	440
Children	9-13 years	700	700
Adolescents	14-18 years	890	890
Adults	19 years and older	900	900
Pregnancy	all ages	-	1,000
Breastfeeding	all ages	-	1,300

TOXICITY

Copper toxicity is rare in the general population. Acute copper poisoning has occurred through the contamination of beverages by storage in copper containing containers as well as from contaminated water supplies (Bremner, 1998). In the U.S. the health-based guideline for a maximum water copper concentration of 1.3 mg/liter is

enforced by the Environmental Protection Agency (EPA) (Fitzgerald, 1998). Symptoms of acute copper toxicity include abdominal pain, nausea, vomiting, and diarrhea, which help prevent additional ingestion and absorption of copper. More serious signs of acute copper toxicity include severe liver damage, kidney failure, coma, and death. Of more concern from a nutritional standpoint is the possibility of liver damage resulting from long-term exposure to lower doses of copper. In generally healthy individuals, doses of up to 10,000 mcg (10 mg) daily have not resulted in liver damage. For this reason, the U.S. Food and Nutrition Board (FNB) recently set the tolerable upper level of intake (UL) for copper at 10 mg/day from food and supplements (Food and Nutrition Board 2001). It should be noted that individuals with genetic disorders affecting copper metabolism (Wilson's disease, Indian childhood cirrhosis, and idiopathic copper toxicosis) may be at risk of adverse effects of chronic copper toxicity at significantly lower intake levels.

Table 1.7
TOLERABLE UPPER INTAKE LEVEL (UL) FOR COPPER
FOOD AND NUTRITION BOARD 2001

Tolerable Upper Intake Level (UL) for Copper	
Age Group	UL (mcg/day)
Infants 0-12 months	Not possible to establish*
Children 1-3 years	1,000
Children 4-8 years	3,000
Children 9-13 years	5,000
Adolescents 14-18 years	8,000
Adults 19 years and older	10,000

*Source of intake should be from food and formula only.

MAGNESIUM

Magnesium plays important role in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, while 6 to 7% is found in other cells, and less than 1% is found outside of cells (Shils, 1997).

Magnesium is an important intracellular cation that is distributed into three major compartments: mineral phase of bones (65%), intracellular space (34%), and extra cellular fluid 1percent (Levine, 1984).

DIETARY SOURCES OF MAGNESIUM

A large national survey indicated that the average magnesium intake for men (about 320 mg/day) and the average intake for women (about 230 mg/day) were significantly below the current recommended dietary allowance (RDA). In men and women over 70 years of age magnesium intakes were even lower (Food and Nutrition Board, 2001). Such findings suggest that marginal magnesium deficiency may be relatively common in the U.S.

Unrefined grains and nuts also have high magnesium content. Meats and milk have intermediate magnesium content, while refined foods generally have the lowest magnesium content. Water is a variable source of intake; harder water usually has a higher concentration of magnesium salts (Food and Nutrition Board, 2001).

FUNCTION

Magnesium is involved in more than 300 essential metabolic reactions, some of which are discussed below (Spencer, 1994 and Shils, 1999).

Energy production

The metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required by the adenosine triphosphate (ATP) synthesizing protein in mitochondria. ATP, the molecule that provides energy for almost all metabolic processes, exists primarily as a complex with magnesium (Mg ATP).

Synthesis of essential molecules

Magnesium is required at a number of steps during the synthesis of nucleic acids (DNA and RNA) and proteins. A number of enzymes participating in the synthesis of carbohydrates and lipids require magnesium for their activity. Glutathione, an important antioxidant, requires magnesium for its synthesis.

Structural roles

Magnesium plays a structural role in bone, cell membranes, chromosomes and ion transport across cell membranes.

Magnesium is required for the active transport of ions like potassium and calcium across cell membranes. Through its role in ion transport systems, magnesium affects the conduction of nerve impulses, muscle contraction, and the normal rhythm of the heart.

Cell signaling

Cell signaling requires Mg ATP for the phosphorylation of proteins and the formation of the cell signaling molecule, cyclic adenosine monophosphate (cAMP). cAMP is involved in many processes, including the secretion of parathyroid hormone (PTH) from the parathyroid glands.

Cell migration

Calcium and magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types. Such affects on cell migration may be important in wound healing.

NUTRIENT INTERACTIONS

Zinc: High doses of zinc in supplement form appear to interfere with the absorption of magnesium. A zinc supplement of 142 mg/day in healthy adult males significantly

decreased magnesium absorption and magnesium balance (the difference between magnesium intake and magnesium loss) (Spencer, 1994).

Fiber: Large increases in the intake of dietary fiber have been found to decrease magnesium utilization in experimental studies. However, the extent to which dietary fiber affects magnesium nutritional status in individuals with a varied diet outside the laboratory is not clear (Shils, 1999 and Food and Nutrition Board, 2001).

Protein: Dietary protein may affect magnesium absorption. One study found that magnesium absorption was lower when protein intake was less than 30 grams/day, and higher protein intakes (93 grams/day vs. 42 grams/day) were associated with improved magnesium absorption in adolescents.

Vitamin D and Calcium: The active form of vitamin D (calcitriol) may increase the intestinal absorption of magnesium to a small extent. However, magnesium absorption does not seem to be calcitriol-dependent as is the absorption of calcium and phosphate. High calcium intake has not been found to affect magnesium balance in most studies. Inadequate blood magnesium levels are known to result in low blood calcium levels, resistance to parathyroid hormone (PTH), and resistance to some of the effects of vitamin- D (Shils, 1999 & Food and Nutrition Board 2001).

DEFICIENCY

Magnesium deficiency in healthy individuals who are consuming a balanced diet is quite rare because magnesium is abundant in both plant and animal foods and because the kidneys are able to limit urinary excretion of magnesium when intake is low. The following conditions increase the risk of magnesium deficiency (Shils, 1997).

Gastrointestinal disorders: Prolonged diarrhea, Crohn's disease, malabsorption syndromes, surgical removal of a portion of the intestine, and intestinal inflammation due to radiation may all lead to magnesium depletion.

Renal disorders (magnesium wasting): Diabetes mellitus and long-term use of certain diuretics may result in increased urinary loss of magnesium.

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Chronic alcoholism: Poor dietary intake, gastrointestinal problems, and increased urinary loss of magnesium may all contribute to magnesium depletion, which is frequently encountered in alcoholics.

Age: Several studies have found that elderly people have relatively low dietary intakes of magnesium. Because intestinal magnesium absorption tends to decrease and urinary magnesium excretion tends to increase in older individuals, suboptimal dietary magnesium intake may increase the risk of magnesium depletion in the elderly (Food and Nutrition Board 2001).

Although severe magnesium deficiency is uncommon, it has been induced experimentally. When magnesium deficiency was induced in humans, the earliest sign was decreased serum magnesium levels (hypomagnesemia). Over time serum calcium levels also began to decrease (hypocalcemia) despite adequate dietary calcium. Hypocalcemia persisted despite increased parathyroid hormone (PTH) secretion. Usually, increased PTH secretion quickly results in the mobilization of calcium from bone and normalization of blood calcium levels. As the magnesium depletion progressed, PTH secretion diminished to low levels. Along with hypomagnesemia, signs of severe magnesium deficiency included hypocalcemia, low serum potassium levels (hypokalemia), retention of sodium, low circulating levels of PTH, neurological and muscular symptoms (tremor, muscle spasms, tetany), loss of appetite, nausea, vomiting, and personality changes (Shils, 1999).

DISEASE TREATMENT AND PREVENTION

Diabetes Mellitus

Magnesium depletion is commonly associated with both insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes mellitus. Between 25% and 38% of diabetics have been found to have decreased serum level of magnesium (hypomagnesemia) (Tosiello, 1996). One cause of the depletion may be increased urinary loss of magnesium as a result of the increased urinary excretion of glucose that accompanies poorly controlled diabetes. Magnesium depletion has been shown to

increase insulin resistance in a few studies and may adversely affect blood glucose control in diabetes.

About one-third of an orally administered load of magnesium is absorbed in the intestines; excretion is primarily via the kidneys, but also via the feces. Hormones that help regulate magnesium levels include calcitonin, parathyroid hormone and insulin. Insulin administration, or insulin released in response to an oral glucose load, has been shown to enhance the uptake of magnesium into cells via an ATPase pump. Magnesium, as an integral part of the activated MgATP complex regulating protein kinases, is directly involved in the control of glucose metabolism, peptide hormone receptor signal transduction, ion channel translocation, stimulus-secretion coupling and stimulus contraction-coupling. Decrements in the enzymatic activities of several metabolic pathways are seen in Diabetes Mellitus as a result of relative magnesium deficiency (Laughlin, 1996).

OTHER DISEASES

Hypertension (high blood pressure)

Large epidemiological studies suggest a relationship between magnesium and blood pressure. However, the fact that foods high in magnesium (fruits, vegetables, whole grains) are frequently high in potassium and dietary fiber has made it difficult to evaluate independent effects of magnesium on blood pressure. A study of more than 30,000 male health professionals found an inverse association between dietary fiber, potassium, and magnesium, and the development of hypertension over a four-year period (Ascherio, 1992). In a similar study of more than 40,000 female registered nurses, dietary fiber and dietary magnesium were inversely associated with systolic and diastolic blood pressures in those who did not develop hypertension over the four-year study period, but neither dietary fiber nor magnesium was related to the risk of developing hypertension (Ascherio, 1996). The Atherosclerosis Risk in Communities (ARIC) study examined the dietary intake and blood levels of magnesium in 7731 men and women and the development of hypertension over a six-year period (Peacock, 1999). The risk of developing hypertension in men and women decreased as serum magnesium levels increased but the trend was only statistically significant in women. Although the investigators found no association

between dietary magnesium and the incidence of hypertension, they suggested that low serum magnesium levels may play a modest role in the development of hypertension.

The results from intervention studies using magnesium supplements to treat hypertension have been conflicting (Food and Nutrition Board 2001). In uncontrolled trials, hypertensive patients on thiazide diuretics experienced decreases in blood pressure when given magnesium supplements. In general, placebo-controlled trials have not been supportive of a blood pressure-lowering effect for magnesium supplementation (Shils, 1999). Modest but significant blood pressure-lowering effects have been reported in two placebo-controlled studies using 485 mg/day of supplemental magnesium in individuals with mild to moderate hypertension for at least 2 months (Witteaman, 1994 and Kawano, 1998), but a number of other studies failed to find any blood pressure-lowering effects with magnesium supplementation. One double blind placebo-controlled study found magnesium supplementation to be beneficial in lowering the blood pressure of individuals with low magnesium status, suggesting that oral magnesium supplementation may be helpful in hypertensive individuals who are depleted of magnesium due to chronic diuretic use, inadequate dietary intake, or both (Shils, 1996).

Preeclampsia-eclampsia (toxemia of pregnancy)

Preeclampsia-eclampsia is a disease that is unique to pregnancy, and may occur anytime after 20 weeks of pregnancy and up to 6 weeks after birth. Approximately 7% of pregnant women in the U.S. develop preeclampsia-eclampsia. Preeclampsia is defined as the presence of elevated blood pressure, protein in the urine, and severe swelling (edema) during pregnancy. Eclampsia occurs with the addition of seizures to the above triad of symptoms. Approximately 5% of women with preeclampsia go on to develop eclampsia, which is a significant cause of maternal death (Crombleholme, 1998). For many years, high dose intravenous magnesium sulfate has been the treatment of choice for preventing eclamptic seizures that may occur in association with preeclampsia-eclampsia late in pregnancy or during labor. Magnesium is believed to relieve cerebral blood vessel spasm, increasing blood flow to the brain (Shils, 1999).

Cardiovascular Diseases

Myocardial infarction (heart attack): Results of a meta-analysis of randomized placebo-controlled trials indicated that an intravenous (IV) magnesium infusion given early after suspected myocardial infarction (MI) could decrease the risk of death. The most influential study included in the meta-analysis was a randomized placebo-controlled trial in 2316 patients that found a significant reduction in mortality (7.8% vs. 10.3% in the placebo group) in those patients who were given intravenous magnesium sulfate within 24 hours of suspected myocardial infarction (Woods, 1992). Follow up from one to five years after treatment revealed that the mortality from cardiovascular disease was 21% lower in the magnesium treated group (Woods, 1994).

A more recent survey of the treatment of more than 173,000 patients with acute MI in the U.S. found that only 5% were given IV magnesium in the first 24 hours after the MI, and that mortality was higher in those patients treated with IV magnesium (Ziegelstein, 2001).

Osteoporosis

Although decreased bone mineral density (BMD) is the primary feature of osteoporosis, other osteoporotic changes in the collagenous matrix and mineral components of bone may result in bones that are brittle and more susceptible to fracture. Magnesium comprises about 1% of bone mineral and is known to influence both bone matrix and bone mineral metabolism. As the magnesium content of bone mineral decreases, bone crystals become larger and more brittle. Some studies have found lower magnesium content and larger bone crystals in bones of osteoporotic women compared to non-osteoporotic controls (Sojka, 1995).

Inadequate serum magnesium levels are known to result in low serum calcium levels, resistance to parathyroid hormone, and resistance to some of the effects of vitamin D, all of which can lead to increased bone loss. A recent study of over 900 elderly men and women found higher dietary magnesium intake to be associated with increased bone mineral density at the hip in both men and women. However, because they are present in

many of the same foods the effect of dietary magnesium could not be separated from the effect of dietary potassium (Tucker, 1999). Few studies have addressed the effect of magnesium supplementation on bone mineral density or osteoporosis in humans. In a small group of postmenopausal women with osteoporosis, magnesium supplementation of 750 mg/day for the first 6 months followed by 250 mg/day months for 18 more months resulted in increased BMD at the wrist after one year, with no further increase after two years of supplementation (Stendig, 1993). Another study found that supplementation with 500 mg/day of magnesium and 600 mg/day of calcium in postmenopausal women who were also taking estrogen replacement therapy and a multivitamin resulted in increased bone density at the heel compared to postmenopausal women receiving only estrogen replacement therapy (Abraham, 1990).

Migraine headaches

Individuals who suffer from recurrent migraine headaches have been found to have lower intracellular magnesium levels (demonstrated in both red blood cells and white blood cells) than individuals who do not experience migraines. Oral magnesium supplementation has been shown to increase intracellular magnesium levels in individuals with migraine, leading to the hypothesis that magnesium supplementation might be helpful in decreasing the frequency and severity of migraine headaches. Two placebo-controlled trials have demonstrated modest decreases in the frequency of migraine headaches after supplementation with 600 mg/day of magnesium compared to placebo (Peikert, 1996 and Mauskop, 1998). However, another placebo-controlled study found no benefit compared to placebo of 485 mg/day of magnesium in reducing the frequency of migraine headaches (Pfaffenrath, 1996). Although no serious adverse effects were noted during these migraine headache trials, the investigators did note adverse effects such as diarrhea and gastric (stomach) irritation in about 20-40% of the individuals taking the magnesium supplements.

SUPPLEMENTS

Magnesium supplements are available as magnesium oxide, magnesium gluconate, magnesium chloride, and magnesium citrate salts, as well as a number of

amino acid chelates, including magnesium aspartate. Magnesium hydroxide is used as an ingredient in a number of antacids (Hendler, 2001).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

In 1997, the Food and Nutrition Board of the Institute of Medicine increased the recommended dietary allowance (RDA) for magnesium, based on the results of recent, tightly controlled balance studies that utilized more accurate methods of measuring magnesium (Food and Nutrition Board, 2001). Balance studies are useful for determining the amount of a nutrient that will prevent deficiency but give little information regarding the amount required for chronic disease prevention or optimum health.

Table 1.8
RECOMMENDED DIETARY ALLOWANCE (RDA) FOR MAGNESIUM
(FOOD AND NUTRITION BOARD 2001)

Recommended Dietary Allowance (RDA) for Magnesium			
Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	30 (AI)	30 (AI)
Infants	7-12 months	75 (AI)	75 (AI)
Children	1-3 years	80	80
Children	4-8 years	130	130
Children	9-13 years	240	240
Adolescents	14-18 years	410	360
Adults	19-30 years	400	310
Adults	31 years and older	420	320
Pregnancy	18 years and younger	-	400
Pregnancy	19-30 years	-	350
Pregnancy	31 years and older	-	360
Breastfeeding	18 years and younger	-	360
Breastfeeding	19-30 years	-	310
Breastfeeding	31 years and older	-	320

TOXICITY

Adverse effects have not been identified from magnesium occurring naturally in food. However, adverse effects from excess magnesium have been observed with intakes of various magnesium salts (supplemental magnesium). The initial symptom of excess magnesium supplementation is diarrhea--a well-known side effect of magnesium that is used therapeutically as a laxative. Individuals with impaired kidney function are at higher risk for adverse effects from magnesium supplementation, and symptoms of magnesium toxicity have occurred in people with impaired kidney function taking moderate doses of magnesium-containing laxatives or antacids. Elevated serum levels of magnesium (hypermagnesemia) may result in a fall in blood pressure (hypotension). Some of the later effects of magnesium toxicity, such as lethargy, confusion, disturbances in normal cardiac rhythm, and deterioration of kidney function, are related to severe hypotension. As hypermagnesemia progresses, muscle weakness and difficulty breathing may occur. Severe hypermagnesemia may result in cardiac arrest (Shils, 1999 & Food and Nutrition Board 2001). The Food and Nutrition Board (FNB) of the Institute of Medicine set the tolerable upper level (UL) for supplemental magnesium intake in generally healthy adolescents and adults at 350 mg/day. This UL represents the highest level of daily supplemental magnesium intake likely to pose no risk of diarrhea or gastrointestinal disturbance in almost all individuals. The FNB cautions that individuals with renal impairment are at higher risk of adverse effects from excess supplemental magnesium intake. However, the FNB also notes that there are some conditions, which may warrant higher doses of magnesium under medical supervision (Food and Nutrition Board 2001).

Table 1.9
TOLERABLE UPPER INTAKE LEVEL (UL) FOR SUPPLEMENTAL
MAGNESIUM (FOOD AND NUTRITION BOARD 2001)

Tolerable Upper Intake Level (UL) for Supplemental Magnesium	
Age Group	UL (mg/day)
Infants 0-12 months	Not possible to establish*
Children 1-3 years	65
Children 4-8 years	110
Children 9-13 years	350
Adolescents 14-18 years	350
Adults 19 years and older	350

*Source of intake should be from food and formula only.

IRON

Iron has the longest and best described history among all the micronutrients. It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes (Beard 1997 and Fairbanks 1999).

DIETARY SOURCES OF IRON

The amount of iron in food (or supplements) that is absorbed and used by the body is influenced by the iron nutritional status of the individual and whether or not the iron is in the form of heme. Because it is absorbed by a different mechanism than nonheme iron, heme iron is more readily absorbed and its absorption is less affected by other dietary factors. Individuals who are anemic or iron deficient absorb a larger percentage of the iron they consume (especially nonheme iron) than individuals who are not anemic and have sufficient iron stores (Yip, 1996 and Lynch, 1997).

Heme iron: Heme iron comes mainly from hemoglobin and myoglobin in meat, poultry, and fish. Although heme iron accounts for only 10-15% of the iron found in the diet, it may provide up to one third of total absorbed dietary iron. The absorption of heme iron is

less influenced by other dietary factors than that of nonheme iron (Beard, 1997 and Lynch, 1997).

Nonheme iron: Plants, dairy products, meat, and iron salts added to foods and supplements are all sources of nonheme iron. The absorption of nonheme iron is strongly influenced by enhancers and inhibitors present in the same meal (Yip, 1996 and Lynch, 1997).

Enhancers of nonheme iron absorption

Vitamin C (ascorbic acid): Vitamin C strongly enhances the absorption of nonheme iron by reducing dietary ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and forming an absorbable iron-ascorbic acid complex.

Other organic acids: Citric, malic, tartaric, and lactic acids have some enhancing effects on nonheme iron absorption.

Meat, fish, and poultry: Aside from providing highly absorbable heme iron, meat, fish, and poultry also enhance nonheme iron absorption. The mechanism for this enhancement of nonheme iron absorption is not clear (Lynch, 1997 & Food and Nutrition Board 2001).

Inhibitors of nonheme iron absorption

Phytic acid (phytate): Phytic acid is present in legumes, grains, and rice and is an inhibitor of nonheme iron absorption. Small amounts of phytic acid (5 to 10 mg) can reduce nonheme iron absorption by 50%. The absorption of iron from legumes, such as soybeans, black beans, lentils, mung beans, and split peas, has been shown to be as low as 2% (Fairbanks, 1999 & Food and Nutrition Board 2001).

Polyphenols: Polyphenols, found in some fruits, vegetables, coffee, tea, wines, and spices, can markedly inhibit the absorption of nonheme iron. This effect is reduced by the presence of vitamin C (Fairbanks, 1999 & Food and Nutrition Board 2001).

Soy protein: Soy protein, such as that found in tofu, has an inhibitory effect on iron absorption that is independent of its phytic acid content (Food and Nutrition Board 2001).

FUNCTION

Oxygen transport and storage

Heme is an iron-containing compound found in a number of biologically important molecules. Hemoglobin and myoglobin are heme-containing proteins that are involved in the transport and storage of oxygen. Hemoglobin is the primary protein found in red blood cells and represents about two thirds of the body's iron. The vital role of hemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen as needed during its circulation through the tissues. Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles (Yip, 1996 and Brody, 1999).

Electron transport and energy metabolism

Cytochromes are heme-containing compounds that are critical to cellular energy production and therefore, life, through their roles in mitochondrial electron transport. They serve as electron carriers during the synthesis of ATP, the primary energy-storage compound in cells. Cytochrome P450 is a family of enzymes that functions in the metabolism of a number of important biological molecules, as well as the detoxification and metabolism of drugs and pollutants. Nonheme iron-containing enzymes, such as NADH dehydrogenase and succinate dehydrogenase are also critical to energy metabolism (Yip, 1996).

Antioxidant and beneficial pro-oxidant functions

Catalase and peroxidases are heme-containing enzymes that protect cells against the accumulation of hydrogen peroxide, a potentially damaging reactive oxygen species (ROS), by catalyzing a reaction that converts hydrogen peroxide to water and oxygen.

As part of the immune response, some white blood cells engulf bacteria and expose them to ROS in order to kill them. The synthesis of one such ROS, hypochlorous acid, by neutrophils is catalyzed by the heme-containing enzyme myeloperoxidase (Yip, 1996 and Brody, 1999).

Oxygen sensing

Inadequate oxygen (hypoxia), such as that experienced by those who live at high altitudes or those with chronic lung disease, induces compensatory physiologic responses, including increased red blood cell formation, increased blood vessel growth (angiogenesis) and increased production of enzymes utilized in anaerobic metabolism. Under hypoxic conditions transcription factors, known as hypoxia inducible factors (HIF), bind to response elements in genes that encode various proteins involved in compensatory responses to hypoxia and increase their synthesis. Recent research indicates that an iron-dependent prolyl hydroxylase enzyme plays a critical role in regulating HIF and consequently, physiologic responses to hypoxia. When cellular oxygen tension is adequate, newly synthesized HIF subunits are modified by a prolyl hydroxylase enzyme in an iron-dependent process that targets HIF for rapid degradation. When cellular oxygen tension drops below a critical threshold, prolyl hydroxylase can no longer target HIF for degradation, allowing HIF to bind to HIF and form an active transcription factor that is able to enter the nucleus and bind to specific response elements on genes (Ivan, 2001 and Jaakkola, 2001).

DNA synthesis

Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis (Fairbanks, 1999 and Beard, 1997). Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune function.

Regulation of intracellular iron

Iron response elements are short sequences of nucleotides found in the messenger RNA (mRNA) that codes for key proteins in the regulation of iron storage and metabolism. Iron regulatory proteins (IRP) can bind to iron response elements and affect

mRNA translation, thereby regulating the synthesis of specific proteins. It has been proposed that when the iron supply is high, more iron binds to IRPs and prevents them from binding to iron response elements on mRNA. When the iron supply is low, less iron binds to IRPs, allowing increased binding of iron response elements. Thus, when less iron is available, translation of mRNA that codes for the iron storage protein, ferritin, is reduced because iron is not available for storage. Translation of mRNA that codes for the key regulatory enzyme of heme synthesis in immature red blood cells is also reduced to conserve iron. In contrast, IRP binding to iron response elements in mRNA that codes for transferrin receptors inhibits mRNA degradation, resulting in increased synthesis of transferrin receptors and increased iron transport to cells (Brody, 1999).

NUTRIENT INTERACTIONS

Vitamin A: Vitamin A deficiency may exacerbate iron deficiency anemia. Vitamin A supplementation has been shown to have beneficial effects on iron deficiency anemia and improve iron status among children and pregnant women. The combination of vitamin A and iron seems to ameliorate anemia more effectively than either iron or vitamin A alone (Lynch, 1997 and Suharno, 1993).

Copper: Adequate copper nutritional status appears to be necessary for normal iron metabolism and red blood cell formation. Anemia is a clinical sign of copper deficiency. Animal studies demonstrate a role for copper in iron absorption (Vulpe, 1999) and iron has been found to accumulate in the livers of copper deficient animals, indicating that copper is required for iron transport to the bone marrow for red blood cell formation (Turnlund JR, 1999).

Zinc: High doses of iron supplements taken together with zinc supplements on an empty stomach can inhibit the absorption of zinc. When taken with food, supplemental iron does not appear to inhibit zinc absorption. Iron-fortified foods have no effect on zinc absorption (Lynch, 1997 & Food and Nutrition Board 2001).

Calcium: When consumed together in a single meal, calcium has been found to decrease the absorption of iron. However, little effect has been observed on serum ferritin levels

(iron stores) with calcium supplement levels ranging from 1,000 to 1,500 mg/day (Lynch, 1997 & Food and Nutrition Board, 2001)

DEFICIENCY

Iron deficiency is the most common nutrient deficiency in the U.S. and the world. Three levels of iron deficiency are generally identified and are listed below from least to most severe (Yip, 1996).

Storage iron depletion: Iron stores are depleted, but the functional iron supply is not limited.

Early functional iron deficiency: The supply of functional iron is low enough to impair red blood cell formation, but not low enough to cause measurable anemia.

Iron deficiency anemia: There is inadequate iron to support normal red blood cell formation, resulting in anemia. The anemia of iron deficiency is characterized as microcytic and hypochromic, meaning red blood cells are measurably smaller than normal and their hemoglobin content is decreased. At this stage of iron deficiency, symptoms may be a result of inadequate oxygen delivery due to anemia and/or sub-optimal function of iron-dependent enzymes. It is important to remember that iron deficiency is not the only cause of anemia, and that the diagnosis or treatment of iron deficiency solely on the basis of anemia may lead to misdiagnosis or inappropriate treatment of the underlying cause (Lee, 1999).

Individuals at increased risk of iron deficiency

Infants and children between the ages of 6 months and 4 years: A full-term infant's iron stores are usually sufficient to last for 6 months. High iron requirements are due to the rapid growth rates sustained during this period (Brody, 1999).

Adolescents: Early adolescence is another period of rapid growth. In females, the blood loss that occurs with menstruation adds to the increased iron requirement of adolescence (Brody, 1999).

Pregnant women: Increased iron utilization by the developing fetus and placenta, as well as blood volume expansion significantly, increases the iron requirement during pregnancy (Brody, 1999).

Individuals with chronic blood loss: Chronic bleeding or acute blood loss may result in iron deficiency. One milliliter of blood with a hemoglobin concentration of 150 grams/liter contains 0.5 mg of iron. Thus, chronic loss of very small amounts of blood may result in iron deficiency. A common cause of chronic blood loss and iron deficiency in developing countries is intestinal parasitic infection. Individuals who donate blood frequently, especially menstruating women, may need to increase their iron intake to prevent deficiency because each 500 ml of blood donated contains between 200 and 250 mg of iron (Fairbanks, 1999).

Individuals with helicobacter pylori infection: H. pylori infection is associated with iron deficiency anemia, especially in children, even in the absence of gastrointestinal bleeding (Sherman, 2001).

Vegetarians: Because iron from plant sources is less efficiently absorbed than that from animal sources, the U.S. Food and Nutrition Board (FNB) has estimated that the bioavailability of iron from a vegetarian diet is only 10%, while it is 18% from a mixed diet. Therefore, the recommended dietary allowance (RDA) for iron from a completely vegetarian diet should be adjusted as follows: 14 mg/day for adult men and postmenopausal women, 33 mg/day for premenopausal women and 26 mg/day for adolescent girls (Food and Nutrition Board 2001).

Individuals who engage in regular, intense exercise: Daily iron losses have been found to be greater in athletes involved in intense endurance training. This may be due to increased microscopic bleeding from the gastrointestinal tract or increased fragility and hemolysis of red blood cells. The FNB estimates that the average requirement for iron may be 30% higher for those who engage in regular intense exercise (Food and Nutrition Board, 2001).

DISEASE TRAETMENT AND PREVENTION

The following health problems and diseases may be prevented through the treatment or prevention of iron deficiency.

Pregnancy complications

Epidemiological studies provide strong evidence of an association between severe anemia in pregnant women and adverse pregnancy outcomes, such as low birth weight, premature birth, and maternal mortality. Iron deficiency can be a major contributory factor to severe anemia, but evidence that iron deficiency anemia is a causal factor in poor pregnancy outcomes is still lacking (Rasmussen, 2001 and Yip, 2000). Nevertheless, most experts consider the control of maternal anemia to be an important part of prenatal health care. Elevated hemoglobin, especially in later pregnancy, is also associated with poor pregnancy outcomes, but there is no evidence that this association is related to high iron intakes or iron supplementation. Rather, elevated hemoglobin in pregnancy is more likely to be explained by underlying conditions like pregnancy induced hypertension or preeclampsia, which are well known to contribute to poor pregnancy outcomes (Yip, 2000).

Impaired immune function

Iron is required by most infectious agents, as well as by the infected host in order to mount an effective immune response. Sufficient iron is critical to several immune functions, including the differentiation and proliferation of T lymphocytes and the generation of reactive oxygen species (ROS) by iron-dependent enzymes, which are used for killing pathogens. During an acute inflammatory response, serum iron levels decrease while levels of ferritin (the iron storage protein) increase, suggesting that sequestering iron from pathogens is an important host response to infection (Beard, 2001). Despite the critical functions of iron in the immune response, the nature of the relationship between iron deficiency and susceptibility to infection, especially with respect to malaria, remains controversial. High-dose iron supplementation of children residing in the tropics has been associated with increased risk of clinical malaria and other infections, such as pneumonia. Studies in cell culture and animals suggest that the survival of infectious agents that spend

part of their life cycle within host cells, such as plasmodia (malaria) and mycobacteria (tuberculosis) may be enhanced by iron therapy. Controlled clinical studies are needed to determine the appropriate use of iron supplementation in regions where malaria is common, as well as in the presence of infectious diseases, such as HIV, tuberculosis, and typhoid (Oppenheimer, 2001).

SUPPLEMENTS

Iron supplements are indicated for the prevention and treatment of iron deficiency. Individuals who are not at risk of iron deficiency (e.g., adult men and postmenopausal women) should not take iron supplements without an appropriate medical evaluation for iron deficiency. A number of iron supplements are available, and different forms provide different proportions of elemental iron. Ferrous sulfate (heptahydrate) is 22% elemental iron; ferrous sulfate (monohydrate) is 33% elemental iron; ferrous gluconate is 12% elemental iron; ferrous fumarate is 33% elemental iron (St. Louis, 2000).

THE RECOMMENDED DIETARY ALLOWANCE (RDA)

The RDA for iron was revised in 2001 and is based on the prevention of iron deficiency and maintenance of adequate iron stores in individuals eating a mixed diet (Food and Nutrition Board 2001).

Table 1.10

**RECOMMENDED DIETARY ALLOWANCE (RDA) FOR IRON
(FOOD AND NUTRITION BOARD 2001)**

Recommended Dietary Allowance (RDA) for Iron			
Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	0.27 (AI)	0.27 (AI)
Infants	7-12 months	11	11
Children	1-3 years	7	7
Children	4-8 years	10	10
Children	9-13 years	8	8
Adolescents	14-18 years	11	15
Adults	19-50 years	8	18
Adults	51 years and older	8	8
Pregnancy	all ages	-	27
Breastfeeding	18 years and younger	-	10
Breastfeeding	19 years and older	-	9

TOXICITY

Overdose: Accidental overdose of iron-containing products is the single largest cause of poisoning fatalities in children under 6 years of age. Although the oral lethal dose of elemental iron is approximately 200-250 mg/kg of body weight, considerably less has been fatal. Symptoms of acute toxicity may occur with iron doses of 20-60 mg/kg of body weight. Iron overdose is an emergency situation because the severity of iron toxicity is related to the amount of elemental iron absorbed. Acute iron poisoning produces symptoms in four stages: (1) Within 1-6 hours of ingestion, symptoms may include nausea, vomiting, abdominal pain, tarry stools, lethargy, weak and rapid pulse, low blood pressure, fever, difficulty breathing, and coma. (2) If not immediately fatal, symptoms may subside for about 24 hours. (3) Symptoms may return 12 to 48 hours after iron ingestion and may include serious signs of failure in the following organ systems: cardiovascular, kidney, liver, hematologic (blood), and central nervous systems. (4) Long-term damage to the central nervous system, liver (cirrhosis), and stomach may

develop 2 to 6 weeks after ingestion (Food and Nutrition Board 2001 and St. Louis, 2000).

Adverse effects: At therapeutic levels for iron deficiency, iron supplements may cause gastrointestinal irritation, nausea, vomiting, diarrhea, or constipation. Stools will often appear darker in color. Iron-containing liquids can temporarily stain teeth, but diluting the liquid helps to prevent this effect. Taking iron supplements with food instead of on an empty stomach may relieve gastrointestinal effects (St. Louis, 2000). The Food and Nutrition Board (FNB) of the Institute of Medicine based the tolerable upper intake level (UL) for iron on the prevention of gastrointestinal distress. The UL for adolescents and adults over the age of 14 years, including pregnant and breastfeeding women is 45 mg/day. It should be noted that the UL is not meant to apply to individuals being treated with iron under close medical supervision. Individuals with hereditary hemochromatosis or other conditions of iron overload, as well as individuals with alcoholic cirrhosis and other liver diseases, may experience adverse effects at iron intake levels below the UL (Food and Nutrition Board, 2001).

Table 1.11
TOLERABLE UPPER INTAKE LEVEL (UL) FOR IRON
(FOOD AND NUTRITION BOARD 2001)

Tolerable Upper Intake Level (UL) for Iron	
Age Group	UL (mg/day)
Infants 0-12 months	Not possible to establish*
Children 1-13 years	40
Adolescents 14-18 years	45
Adults 19 years and older	45

*Source of intake should be from food and formula only.

THE OBJECTIVE OF THE PRESENT WORK

To enrich some vegetables through their roots with some selected trace minerals:-

- That may act as hypoglycemic agents in the treatment of diabetes.
- May be helpful in dietary deficiency diseases.
- Would reduce serving size of vegetables and increase dietary intake of these minerals

CHAPTER- 2

MATERIALS AND METHODS

PREPARATION OF SOIL

A site was selected for sowing seeds of experimental vegetables in the premises of Pakistan Council of Scientific and Industrial Research, Laboratories Complex, Peshawar. This was fully exposed to sun-light and never used for growing vegetables or any other crops before. Before sowing seeds, the soil was cultivated to a depth of more than one foot, removed stones and broken large clods until a smooth and fine texture reached.

A plot was prepared having three columns A, B, C and seven rows for both winter and summer vegetables in their respective seasons (Fig. 2.1 & 2.2). In main plots each row composed of three boxes (Box-A, Box-B and Box-C), which were further divided in to four rectangular sub-boxes (A-1, A-2, A-3, A-4, B-5, B-6, B-7, B-8 and C-9, C-10, C-11, C-12) respectively. Thus one row had twelve sub-boxes and sub-box (C-12) was kept as control. Moreover, the sub-boxes from 1 to 11 represent one sample which was supplemented either with individual element or combination of elements. The dimensions of each sub-box were; length 1.25 feet and width 1 foot. There was 1/2 foot distance between each sub-box with in the main box, while a distance of approximately 1 foot in case of main boxes. Boxes were separated with polyethylene plastic to avoid water and mineral penetration.

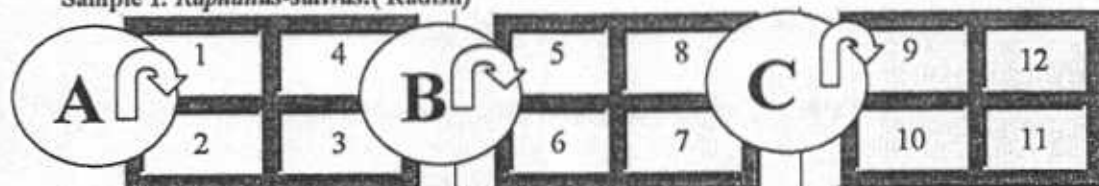
The plot design for two summer vegetables bitter gourd and Bath sponge was a little modified by leaving some extra space for spreading stems.

Before sowing seeds, soil samples were taken by combination of five drills (0-15cm, 15-30cm & 30-45cm) from the corners and center of the plots. Soil samples were air dried in shade, ground with a wooden mortar and sieved through a 2mm nylon mesh size sieve. The samples were then tightly packed in polythene bags and labeled for further analysis.

The reason for taking samples from the plot prepared for summer vegetables in advance was; 1) the summer and winter plots were in close proximity 2) moreover, the summer plot area was protected by polyethylene sheet mounted on wooden frame to avoid any compositional changes in case of rain.

Figure 2.1 DIAGRAMMATIC REPRESENTATION OF THE SOIL PLOT USED FOR GROWING WINTER VEGETABLES.

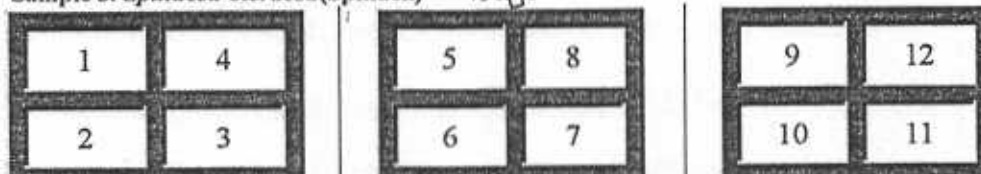
Sample 1. *Raphanus-sativus*. (Radish)



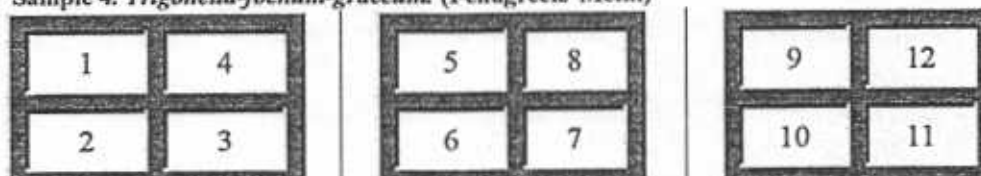
Sample 2. *Brassica-rapa* (Turnip)



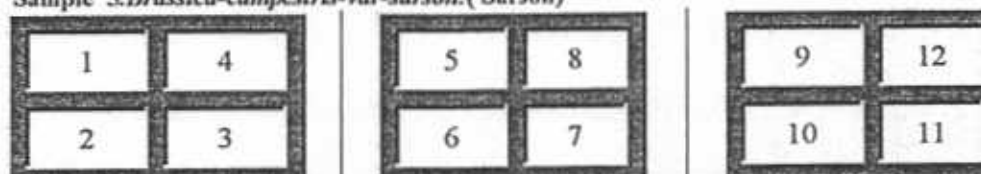
Sample 3. *Spinacea-oleracea* (Spinach) $\frac{1}{2}$ ft



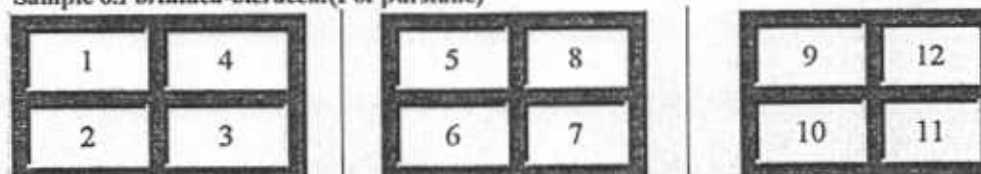
Sample 4. *Trigonella-foenum-graceum*. (Fenugreek/ Methi)



Sample 5. *Brassica-campestris-var-sarson*. (Sarson)



Sample 6. *Portulaca-oleracea*. (Pot purslane)



Sample 7. *Allium-sativum*. (garlic)

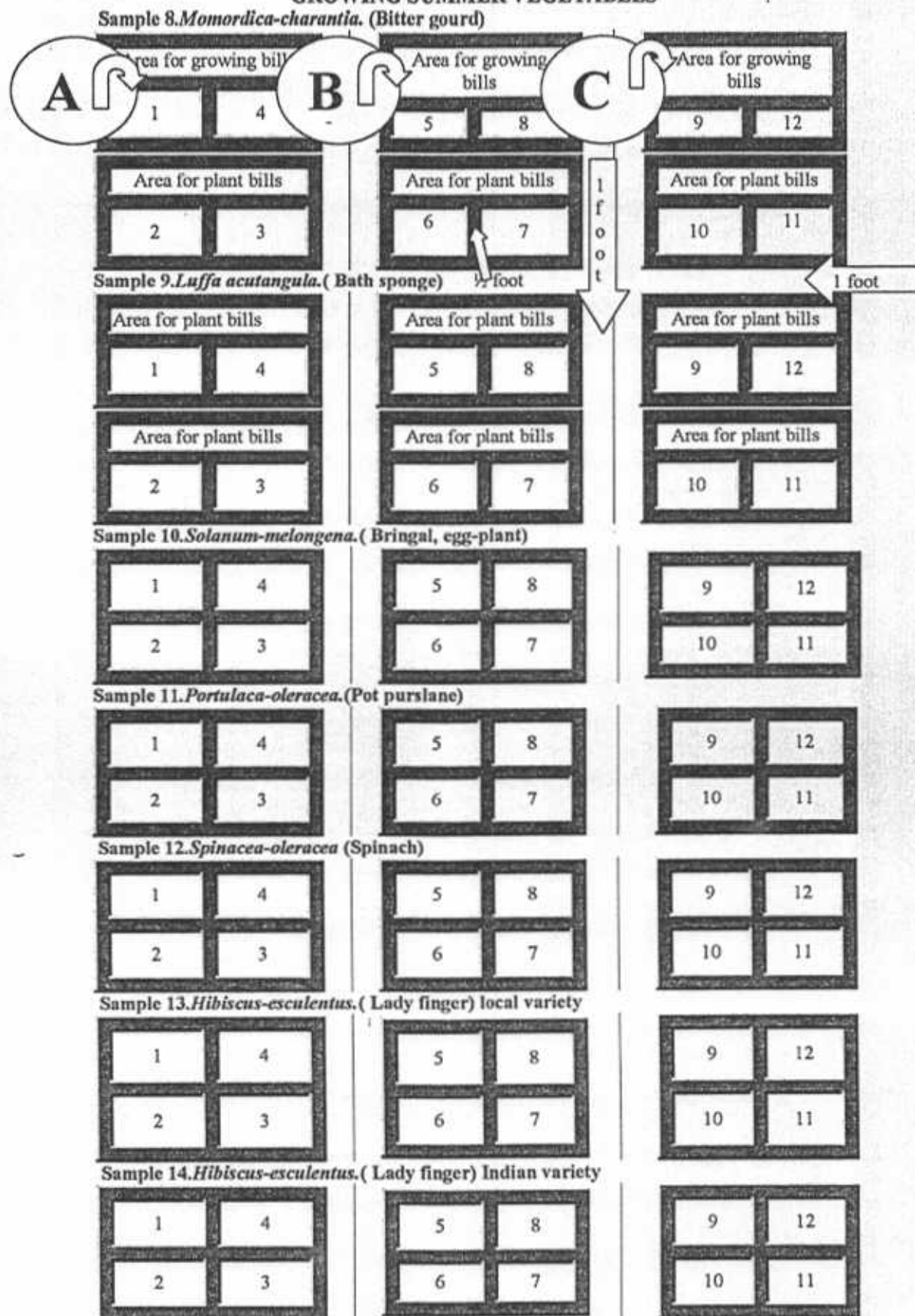


The dimension of each sub-box is; length 1.25feet, & width 1foot .There is $\frac{1}{2}$ foot distance among each sub-box with in the main box while a distance of approximately 1 foot in case of main boxes.

Box-A Sub- boxes:	Supplementation of elements	Box-B Sub- boxes:	Supplementation of elements	Box-C Sub- boxes:	Supplementation of elements
A-1	Com-1. Cr, Cu, Mg	B-5	Ind-5. Cr	C-9	Ind-9. Cr
A-2	Com-2. Cr, Mn, Zn	B-6	Ind-6. Zn	C-10	Ind-10. Zn
A-3	Com-3. Cr, Fe, Zn	B-7	Ind-7. Mg	C-11	Ind-11. Mg
A-4	Com-4. Cr, Cu, Mg, Zn, Mn, Fe	B-8	Com-8. Cr, Mg, Zn	C-12	Control

Com =combination, Ind= Individual

Figure 2.2 DIAGRAMMATIC REPRESENTATION OF THE SOIL PLOT USED FOR GROWING SUMMER VEGETABLES



The dimension of each sub-box is; length 1.25feet, & width 1foot .There is $\frac{1}{2}$ foot distance among each sub-box with in the main box while a distance of approximately 1 foot in case of main boxes

CULTIVATION OF WINTER AND SUMMER SEASON VEGETABLES

Purchasing of Seeds

Fresh seeds packed in plastic bags were purchased from local market for winter and summer vegetables in their respective seasons. The seeds were physically healthy, normal in size, free of foreign seeds and disease.

Planting and Fertilizing Seeds

Radish, turnip, spinach, fenugreek, sarson, pot-purslane and lady-finger seeds were planted about 1/2-inch deep in the soil. In case of radish, turnip and lady-finger, three seeds 4-inch apart in a triangular form were placed in each sub-box. While two dozen seeds of each, spinach, fenugreek, sarson and pot-purslane were sown 1-inch apart in three rows with eight seeds per row. For planting garlic, its healthy and large cloves were purchased from grocery store. Cloves were separated by prying apart and sown two cloves about 3-inches deep in the soil with pointed end up. For planting eggplant, its seedlings were purchased from nursery. One seedling was transplanted in each sub-box.

Bitter gourd and Bath sponge seeds were planted in a similar manner. As bitter gourd seeds are very hard and needs to be softened before sowing. Therefore, the seeds were soaked in distilled water for 24 hours before sowing, while bath sponge seeds were sown with out soaking. Thus in each case two seeds were placed 1-inch deep in the soil with pointed end up in the sub-boxes.

Application of Fertilizer

As a general rule for best results, phosphorous, potassium and nitrogen fertilizers are applied before or at the time of planting. Fertilizer needs were determined after performing complete soil tests. On the basis of soil test results there was no need for K fertilizer, while P and N were required in minute quantity. For this purpose 4 gram of Diammonium phosphate (DAP) fertilizer per box was used.

Application Method of Fertilizer

The fertilizer was broadcast on the surface and then watered into soil.

Application Pattern of Fe, Zn, Mn, Cr, Mg, and Cu to Soil

0.1Molar solution of each Fe, Zn, Mn, Cr, Mg, and Cu was prepared. Different concentration of Fe, Mn, Cr, Mg and Cu were applied to roots of each experimental vegetable in the soil either individual element or in combination at different stages as;

First Application

Box-A	Supplementation of elements	Box-B	Supplementation of elements
Sub-boxes:		Sub-boxes:	
A-1	Com-1. Cr, Cu, Mg	B-5	Ind-5. Cr
A-2	Com-2. Cr, Mn, Zn	B-6	Ind-6. Zn
A-3	Com-3. Cr, Fe, Zn	B-7	Ind-7. Mg
A-4	Com-4. Cr, Cu, Mg, Zn, Mn, Fe	B-8	Com-8. Cr, Mg, Zn

Second Application

Box-C	Supplementation of elements
Sub-boxes:	
C-9	Ind-9. Cr
C-10	Ind-10. Zn
C-11	Ind-11. Mg
C-12	Control

Com =combination, Ind= Individual

For supplementation of required amount of these elements to vegetables, specified volume was taken (shown in Table 2.1) in a one liter beaker ,added tap water and this was then spread around 1"-2" area of roots of vegetables. The beaker was rinsed many times with approximately three litters more water to ensure the complete transfer of elements. The soil was not watered for a week prior this application, so that proper penetration of the mineral solution in the soil could be achieved.

Table 2.1**Amount of Fe, Zn, Mn, Cr, Mg, and Cu Taken for Application to Experimental Plants**

Element	Concentration of Element Per Milliliter of 0.1 M Solution. (mg/ml)	Volume Taken of 0.1 Molar Solution (ml)	Volume Taken Containing Total Concentration (mg) of Element
Fe	5.585	10	55.85mg
Zn	6.537	25	163.425mg
Mn	5.494	10	54.94 mg
Cr	5.2	6	31.2 mg
Mg	6.354	5	38.124 mg
Cu	2.430	50	121.5 mg

Watering Plants

The winter vegetables were watered 14 liters per sub-box once a week and summer vegetables twice a week that is 20 liters per week. The water was applied very slowly through bucket in intervals to prevent water runoff from the box and to ensure maximum penetration. The approximate water applied to each plant from start to harvest is given (Tables 2.2 and 2.3).

Care Through out growing period

To protect the plants from any rain water, a wooden frame was constructed on the plot to cover with polyethylene plastic in case of rain.

Harvesting

After harvesting vegetables were brought to the laboratory and washed with tap water to remove the soil followed three times with distilled water. Samples were cut into small pieces with plastic knife before oven drying at 70 °C until the weight became stable. Samples were then ground with mortar and 0.5g of each sample was wet digested with HNO₃: HClO₄ (2:1) for 2-3 hrs on heating mantle. (AOAC,1984).Digested samples were filtered through 0.45 µm pore size Millipore filter and volume was made to 100 ml with

distilled water. Trace minerals concentration was determined on Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer equipped with standard hollow cathode lamps as radiation source and air acetylene flames was used for absorption measurement of elements. The elements analyzed were Cr, Mn, Zn, Cu and Fe and Mg.

SOIL ANALYSIS

Soil samples were analyzed for soil pH, electrical conductivity (EC), texture, organic matter, lime content (CaCO_3), Potassium, Phosphorus and extractable heavy metals.

a. pH and Electrical Conductivity (EC)

The pH and EC of the soil samples were determined in 1:1 soil and water suspension by pH meter and Conductivity meter respectively at 25 °C (Richard, 1960).

b. Texture

By Bouyoucos Hydrometer method using Na_2CO_3 as dispersing agent (Kochler, 1984)).

c. Organic Matter

By Walkly and Black method as described by Jackson (Jackson, 1958).

d. Lime content (Ca CO_3)

By the acid neutralization method according to Black (Black, 1965).

e. POTASSIUM (K)

By Flame Photometer.

f. PHOSPHOROUS (P)

By Rapid and sensitive colorimetric method Using spectrophotometer.

g. DTPA-Extractable Heavy Metals

Extractable Heavy metals in each soil sample were estimated with the help of Atomic Absorption Spectrophotometer Model Hitachi Polarized Z-8000 Japan by using NH_4HCO_3 -DTPA extracting solution. 20 ml NH_4HCO_3 -DTPA (diethylene triamine penta acetic acid) extracting solution was added to exactly 10 g weighed air dried soil sample and shaken for 15 minutes. After shaking, the suspension was filtered through whatman filter paper No. 42. The filtrate was then analyzed for extractable, Cu, Fe, Zn, Cr, Mg, and Mn (Halvin, 1981).

WATER ANALYSIS (APHA, 1998)

Tap water used for irrigation purpose was analyzed only for:

a. pH and Electrical Conductivity (EC)

The pH and EC of the water sample was determined by pH meter and Conductivity meter respectively at 25 °C.

b. Chloride as Cl^{-1}

By Titrimetric method.

c. Sulphate as SO_4

By Titrimetric method.

d. Trace Metals

By Atomic Absorption Spectrophotometer.

FERTILIZERS

Fertilizers applied were;

a. DAP (Diammonium Phosphate)

Chemical Formula:	$(\text{NH}_4)_2 \text{HPO}_4$
Composition:	(18.46.0) 18 % N, 46 % P_2O_5 or 20 % P
Make:	United States of America (USA) Imported and Marketed By 7-Stars United Agro. Chemicals (UAC) Pakistan.

b. Minerals Salts (Alam. S.M.2001)

1. Ferrous Sulfate (Sigma)

Formula:	$(\text{Fe SO}_4.7\text{H}_2\text{O})$
Formula weight:	278.0
Purity:	99.0 %
Insoluble matter	$\leq 0.01\%$
Chloride	$\leq 0.001\%$
Phosphate (PO_4)	$\leq 0.001\%$
Copper (Cu)	$< 0.005\%$
Ferric Iron (Fe^{+3})	74 0.1%
Manganese (Mn)	$\geq 0.05\%$
Substances not precipitated by ammonium hydroxide	$\leq 0.05\%$
Zinc (Zn)	$\leq 0.005\%$

2. Zinc Sulfate (Merck) extra pure.

Formula:	$(\text{Zn SO}_4.7\text{H}_2\text{O})$
Formula weight:	287.54

3. Manganese Sulphate (Sigma)

Formula:	$(\text{Mn SO}_4.\text{H}_2\text{O})$
Formula weight:	169.0
Purity:	98.0-101.0%
Insoluble matter	$\leq 0.01\%$
Loss on ignition	$\leq 10.0-12.0\%$
Chloride	$\leq 0.005\%$
Calcium	$\leq 0.005\%$
Heavy metals (as Pb)	$\leq 0.002\%$
Iron (Fe)	$\leq 0.002\%$
Magnesium (Mg)	$\leq 0.005\%$

Nicle (Ni)	≤0.02%
Potassium (K)	≤0.01%
Sodium (Na)	≤0.05%
Zinc (Zn)	≤0.005%

**4. Chromium (III)-chloride pure crystal
(RieDel-De-Haen AG. Seelze-Hannover)**

Formula:	$[\text{CrCl}_2 (\text{H}_2\text{O})_4] \text{ Cl} \cdot 2\text{H}_2\text{O}$
Formula weight:	266.34
Made:	Germany

5. Magnesium Sulfate (Merck) extra pure

Formula:	$\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$
Formula weight:	46.47
Purity:	99.0-100.5%
Chloride	< 0.014%
Lead (Pb)	< 0.0005%
Arsenic (As)	< 0.0002%
Calcium (Ca)	< 0.01%
Iron (Fe)	< 0.001%
Selenium (Se)	< 0.001%

6. Copper Sulfate (Sigma)

Formula:	$\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$
Formula weight:	249.7
Purity:	98.0-102.0%
Insoluble matter	≤ 0.005%
Chloride	≤ 0.001%
Nitrogen compounds as Nitrogen	≤ 0.001%
Substances not precipitated by hydrogen sulfide (as sulfates)	≤ 0.1%
Iron (Fe)	≤ 0.003%
Ammonium sulfide metals other than iron	≤ 0.00

DATA ANALYSIS

The data was presented for triplicate measurement in each case by Student's t test. Results were expressed as mean ± Standard Error (SE) for each element. Comparison was made with 0.05 level of significant. The result with less coefficient of variance would be considered more consistent. Each individual result for which P-value was less would be considered highly significant. Calculations were performed using MSTAT-C statistical programme

Table 2.2
Winter Vegetables Schedule of Sowing, Trace Minerals Application and Harvesting

S.No	Name of Sample	Date of Sowing	1 st Application Column A & B	2 nd Application Column C	Date of Harvesting	Total Days till Harvesting	Approximate Volume of Water in Liters Applied to Plants till Harvesting
1.	Spinacea-oleracea(Spinach)*	15.10.03	25.10.03	09.11.03	26.11.03	42	84
2.	Trigonella-foenum-graceum. (Fenugreek/Methi)*	15.10.03	25.10.03	09.11.03	20.12.03	66	132
3.	Brassica-campestris-var-sarson. (Sarson)*	15.10.03	25.10.03	09.11.03	25.11.03	41	82
4.	Portulaca-oleracea. (Pot purslane)*	15.10.03	25.10.03	09.11.03	26.11.03	42	84
5.	Raphanus-sativus. (Radish)**	15.10.03	04.11.03	04.12.03	13.01.04	90	180
6.	Brassica-rapa (Turnip) **	15.10.03	04.11.03	04.12.03	27.12.03	73	146
7.	Allium-sativum. (garlic)***	15.10.03	14.11.03	29.12.03	31.03.04	167	270

* Supplementation with interval after 10days of sowing. (Supplementation gap between 1st and 2nd application was 15 days)

** Supplementation with interval after 20days of sowing. (Supplementation gap between 1st and 2nd application was 30 days)

*** Supplementation with interval after 30days of sowing. (Supplementation gap between 1st and 2nd application was 45 days)

Table 2.3
Summer Vegetables, Schedule of Sowing, Trace Minerals Application and Harvesting

S. No	Name of Sample	Date of Sowing	1 st Application Column A & B	2 nd Application Column C	Date of Harvesting	Total Days till Harvesting	Approximate Volume of Water in Liters Applied to Plants till Harvesting
1.	Portulaca-oleracea (Pot purslane)*	30.03.04	13.04.04	23.04.04	04.05.04	35	100
2.	Spinacea-oleracea (Spinach)*	30.03.04	13.04.04	23.04.04	04.05.04	35	100
3.	Momordica-charantia (Bitter gourd)**	30.03.04	18.04.04	08.05.04	29.05.04	60	170
4.	Luffa acutangula (Bath sponge)**	30.03.04	18.04.04	08.05.04	24.05.04	55	160
5.	Solanum-melongena (Bringal, egg-plant)**	30.03.04	18.04.04	08.05.04	24.05.04	55	160
6.	Hibiscus-esculentus. (Lady finger) local variety.**	30.03.04	18.04.04	08.05.04	26.05.04	57	165
7.	Hibiscus-esculentus. (Lady finger) Indian variety.**	30.03.04	18.04.04	08.05.04	26.05.04	57	165

* Supplementation with interval after 13days of sowing. (Supplementation gap between 1st and 2nd application was 10 days)

** Supplementation with interval after 18days of sowing. (Supplementation gap between 1st and 2nd application was 20 days)

Table 2.4
The Instrumental Conditions Maintained For Each Metal on Atomic Absorption Spectrometer Model Hitachi Polarized Z-8000 Japan

S#	Metals	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Burner Height (mm)	Oxidant (Air) Kg/cm ²	Fuel (Acetylene) pressure Kg/cm ²
1.	Cr	359.3	1.3	7.5	7.5	1.60	0.40
2.	Mn	279.5	0.4	7.5	7.5	1.60	0.30
3.	Zn	213.8	1.3	10.0	7.5	1.60	0.20
4.	Cu	324.8	1.3	7.5	7.5	1.60	0.30
5.	Fe	248.3	0.2	10.0	7.5	1.60	0.30
6.	Mg	285.2	1.3	7.5	7.5	1.60	0.20

CHAPTER -3

RESULTS

SOIL AND WATER

The chemical analysis and trace metal results of soil and water are given in Table 3.1(a) to 3.1(d).

Table 3.1

a) Chemical Analysis of Soil

Soil sample	Textural Class			Lime content CaCO ₃ %	Organic Matter %	P (mg/Kg)	K (mg/Kg)	pH	EC dS/m
	Clay %	Silt %	Sand %						
Depth (0-15cm)	28.8	32.0	39.2	12.66	0.92	7.23	208	7.4	0.10
Depth (15-30 cm)	28.8	28.0	43.2	13.33	1.19	6.66	192	7.3	0.11
Depth (30-45 cm)	28.0	28.4	43.6	13.45	1.18	6.32	185	7.3	0.11
Depth (0-45cm)	28.53	29.46	42	13.15	1.10	6.74	195	7.3	0.12
Average									

b) Chemical Analysis of Water

Sample	pH	Electrical Conductivity dS/m	Chloride as Cl ⁻¹ (mg/L)	Sulphate as SO ₄ (mg/L)	Potassium as K ⁺ (mg/L)
Water	7.2	0.38	31.14	30.19	3.2

c) Trace Metals in Soil (mg/Kg) Dry Weight Basis

Soil sample	Cr	Zn	Mn	Cu	Mg	Fe
Depth (0-15cm)	2.42±0.006 C.V(%)=0.41 P-Value=0.0000	5.00±0.011 C.V(%)=0.40 P-Value=0.0000	20.69±0.115 C.V(%)=0.96 P-Value=0.0000	6.90±0.023 C.V(%)=0.57 P-Value=0.0000	35.30±0.202 C.V(%)=0.99 P-Value=0.0000	39.00±0.231 C.V(%)=1.02 P-Value=0.0000
Depth (15-30 cm)	2.40±0.006 C.V(%)=0.41 P-Value=0.0000	4.26±0.006 C.V(%)=0.23 P-Value=0.0000	19.54±0.087 C.V(%)=0.76 P-Value=0.0000	5.40±0.006 C.V(%)=0.18 P-Value=0.0000	33.82±0.115 C.V(%)=0.59 P-Value=0.0000	38.92±0.173 C.V(%)=0.77 P-Value=0.0000
Depth (30-45 cm)	2.44±0.011 C.V(%)=0.82 P-Value=0.0000	4.50±0.017 C.V(%)=0.66 P-Value=0.0000	19.37±0.069 C.V(%)=0.62 P-Value=0.0000	5.20±0.006 C.V(%)=0.19 P-Value=0.0000	32.86±0.086 C.V(%)=0.45 P-Value=0.0000	38.46±0.144 C.V(%)=0.65 P-Value=0.0000

d) Trace Metals in Water (mg/L)

Sample	Cr	Zn	Mn	Cu	Mg	Fe
Water	0.06±0.000 C.V(%)=0.000 P-Value=0.000	0.07±0.006 C.V(%)=14.3 P-Value=0.0072	0.02±0.000 C.V(%)=0.000 P-Value=0.000	0.05±0.000 C.V(%)=0.000 P-Value=0.000	21.81±0.040 C.V(%)=0.32 P-Value=0.0000	2.39±0.011 C.V(%)=0.83 P-Value=0.0000

SOIL

Soil Texture

The result of the soil texture revealed that it was mainly Clay-loam. (Table. 3.1 a)

Nitrogen

Nitrogen (N) fertilizer recommendations for vegetables and flower gardens are based on the organic matter content of the soil and the crops that are grown. The results obtained for organic matter of different depths; 0.92% (0-15cm); 1.19% (15-30cm) and 1.18% (30-45cm) in which the top soil was having less organic matter than the mid and bottom depths. In case the soil test value for organic matter is above 1.00%, then 60kg/ha nitrogen (N) is recommended (Rehman, 2001).

Phosphorus and Potassium

Phosphorus results obtained for different depths were in the order of; 7.23ppm (0-15cm); 6.66ppm (15-30cm) and 6.32ppm (30-45cm) with an average of 6.74ppm. Likewise, Potassium results for different depths were; 206ppm (0-15cm); 192ppm (15-30cm) and 185ppm (30-45cm) with an average of 195ppm. If the soil's test Phosphorus (P) level is 5-10ppm then phosphorus (P_2O_5) 60kg/ha is recommended. Similarly if the soil test Potassium (K) level is above 150 then no K_2O is required (Rehman, 2001).

Lime content ($CaCO_3$)

The lime content ($CaCO_3$) at different depths found was: 12.66% (0-15cm); 13.33% (15-30 cm) and 13.45% (30-45 cm) with an average of (13.15%). The lime content at different depths was almost the same. Generally the lime content of NWFP soils ranges 3.85 to 24.2 % (Bhatti, 1997).

pH

pH of soil at different depth level was; 7.4 (0-15cm); 7.3 (15-30cm) and 7.3 (30-45cm), with an average of pH 7.3, which is considered as neutral or slightly alkaline. In acidic soil Cd, Hg, Ni and Zn are relatively mobile while As, Be and Cr are moderately mobile and Cu, Pb and Se are slowly mobile (Fulter, 1977).

Electrical Conductivity (EC)

The electrical conductivity (EC) at different depths was in the order of 0.10 dsm^{-1} (0-15cm); 0.11 dsm^{-1} (15-30cm) and 0.11 dsm^{-1} (30-45cm) whereas the average EC was 0.12 dsm^{-1} . The electrical conductivity is the same in all the three depths showing the uniformity of free cations and anions like Na^+ , K^+ , Cl^- , NO_2^- etc. The soil is non-saline and such soils present no harm to agricultural crops (Maas and Hoffman, 1986).

Soil Minerals

The concentrations of Cr, Zn, Mn, Cu, Mg and Fe found in soil on dry weight basis at different depths were: 2.42 ± 0.006 , 5 ± 0.011 , 20.69 ± 0.115 , 6.90 ± 0.023 , 35.30 ± 0.202 and $39.00 \pm 0.231 \text{ mg/kg}$ at (0-15cm) 2.40 ± 0.006 , 4.26 ± 0.006 , 19.54 ± 0.087 , 5.40 ± 0.006 , 33.82 ± 0.115 and $38.92 \pm 0.173 \text{ mg/Kg}$ at (15-30 cm); 2.44 ± 0.011 , 4.50 ± 0.017 , 19.37 ± 0.069 , 5.20 ± 0.006 , 32.86 ± 0.086 and $38.46 \pm 0.144 \text{ mg/Kg}$ at (30-45cm). (Table 3.1 c)

Thus according to Halvin and Sultanpour(1981), the results indicates that the soil was adequate in Cu, Fe, Mn, and Zn in terms of their concentrations for agricultural crops.

WATER

pH

As pH of the water determined (7.2) was almost same that of soil pH 7.3, hence pose no effect to the soil pH. (Table 3.1 b)

Electrical Conductivity (EC)

The electrical conductivity (EC) of the water determined was 0.38 dS/m. It was different to EC of soil which is mainly due to the number of free cations and anions like Na^+ , K^+ , Cl^- , NO_2^- etc.

Chloride

The Chloride contents of the water were; 31.14 mg/L. Which is quite low. Crops are sensitive to high chloride contents. For chlorides there is a maximum limit of (100 mg/L) for irrigation water and above this limit is dangerous for crops (USEPA, 1985).

Sulphate

The amount of sulphate was 30.19 mg/L. where as the recommended limit for sulphate is (250 mg/L) (USEPA, 1985).

RESULTS OF WINTER AND SUMMER SEASON VEGETABLES

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in milligrams per Kilogram (mg/Kg) dry weight basis for each winter vegetable sample has been shown in Table 3.2 to 3.8 (Figures 3.2 to 3.8) and summer vegetables in Table 3.9 to 3.15 (Figures 3.9 to 3.15).

Similarly winter vegetable samples with maximum concentration and increase of Cr, Zn, Mn, Cu, Mg and Fe (mg/Kg) has been shown in Tables 1a & 1b (Figures 1a and 1b) and summer vegetables in Tables 2a and 2b (Figures 2a and 2b).

WINTER SEASON VEGETABLES

1. *Raphanus-sativus*. (Radish Roots and Leaves)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples of roots observed was $(8\pm0.057, 58\pm0.144, 44\pm0.231, 6\pm0.034, 2264\pm5.780$ and 568 ± 3.468 with a maximum concentration of Cr, Zn, Cu and Mg $14\pm0.115, 120\pm1.156, 30\pm0.289$ and 2476 ± 15.02) mg/Kg on dry weight basis in sub-boxes (C-9), (B-6), (A-1) and (A-2) respectively. The concentration of Mn and Fe did not exceed in any sub-box with respect to control sample. The total increase of Cr, Zn, Cu and Mg recorded was (6, 62, 24 and 212) mg/Kg dry weight basis. While in sub-boxes (B-6), (C-11), (B-8), (B-6), (A-3) and (A-1) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe ($6\pm0.028, 46\pm0.231, 36\pm0.104, 4\pm0.005, 1878\pm5.780$ and 384 ± 4.624 with total decrease of (2, 12, 8, 2, 386 and 184) mg/Kg dry weight basis was observed (Table 3.2a and Figure 3.2a).

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples of leaves observed was ($10\pm0.115, 122\pm1.156, 192\pm1.156, 12\pm0.046, 2960\pm33.52$ and 2198 ± 9.248 with a maximum concentration of Cr, Zn, Cu and Mg ($16\pm0.231, 16\pm0.219, 16\pm0.231, 166\pm0.809, 44\pm0.462$ and 3050 ± 9.248) mg/Kg on dry weight basis in sub-boxes (A-4, B-5, C-9), (B-6), (A-1) and (C-11) respectively. The concentration of Mn and Fe in leaves also did not exceed in any sub-box with respect to control sample. The total increase of Cr, Zn, Cu and Mg in sub-boxes (A-4, B-5, C-9), (B-6), (A-1) and (C-11) recorded was (6, 44, 32 and 90) mg/Kg dry weight basis. While in sub-boxes (B-5, C-11), (A-4), (B-7), (A-4) and (A-4) minimum concentration of Zn, Mn, Cu, Mg and Fe ($104\pm0.520, 104\pm0.578, 110\pm1.156, 6\pm0.023, 2244\pm8.092$ and 1236 ± 8.092 with total decrease of (18, 82, 6, 716 and 962) mg/Kg dry weight basis was observed (Table 3.2b and Figure 3.2b).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application.

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Overall the concentration was increased in all sub-boxes except with no increase and decrease in sub-boxes (A-3, B-7, C-10) of radish roots and (A-1, B-6, C-10, C-11) leaves respectively. Marked increase was observed in sub-boxes (C-9) of radish

roots at 2nd stage and sub-boxes (A-4, B-5 and C-9) of radish leaves at both 1st and 2nd stage of supplementation.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Increase was observed in all sub-boxes of both radish roots and leaves supplemented with combinations. Marked increase was recorded in sub-boxes (B-6) of both radish roots and leaves at 1st stage.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Decrease was observed in magnesium concentration in all sub-boxes except with an increase in sub-box (A-1) of radish roots at 1st stage and sub-boxes (C-11) of both radish roots and leaves at 2nd stage. The reason of this increase in magnesium content at later stage is due to increase input of Mg through irrigation water having high concentration of Magnesium.

Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. The copper concentration was increased in sub-boxes (A-1, A-4), while manganese and iron concentration in sub-boxes (A-2, A-4) and (A-3, A-4) was decreased both in radish roots and leaves.

Table 3.2a *Raphanus-sativus* (Radish Roots) Concentration of Cr, Zn, Mn, Cu, Mg and Fe (mg/Kg) Dry Weight Basis and Effect on their Uptake when supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or In Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	10±0.115 C.V(%)=2.00 P-Value=0.0001	46±0.462 C.V(%)=1.73 P-Value=0.0001	42±0.231 C.V(%)=0.95 P-Value=0.0001	30±0.289 C.V(%)=1.66 P-Value=0.0001	2298±10.40 C.V(%)=0.78 P-Value=0.0001	384±4.624 C.V(%)=2.08 P-Value=0.0001	↑	↓	↓	↑	↑	↓
A-2	10±0.115 C.V(%)=2.00 P-Value=0.0001	72±0.289 C.V(%)=0.69 P-Value=0.0001	38±0.208 C.V(%)=0.94 P-Value=0.0001	10±0.052 C.V(%)=0.90 P-Value=0.0001	2476±15.02 C.V(%)=1.05 P-Value=0.0001	486±2.312 C.V(%)=0.82 P-Value=0.0001	↑	↑	↓	↑	↑	↓
A-3	8±0.052 C.V(%)=1.12 P-Value=0.0000	64±0.115 C.V(%)=0.31 P-Value=0.0001	40±0.173 C.V(%)=0.75 P-Value=0.0001	6±0.034 C.V(%)=1.00 P-Value=0.0001	1878±5.780 C.V(%)=0.53 P-Value=0.0001	464±2.890 C.V(%)=1.07 P-Value=0.0001	N	↑	↓	N	↓	↓
A-4	10±0.115 C.V(%)=2.00 P-Value=0.0001	78±0.346 C.V(%)=0.76 P-Value=0.0001	38±0.156 C.V(%)=0.71 P-Value=0.0001	24±0.086 C.V(%)=0.62 P-Value=0.0001	2188±12.71 C.V(%)=1.00 P-Value=0.0001	422±3.468 C.V(%)=1.42 P-Value=0.0001	↑	↑	↓	↑	↓	↓
B-5	10±0.115 C.V(%)=2.00 P-Value=0.0001	50±0.115 C.V(%)=0.40 P-Value=0.0001	38±0.115 C.V(%)=0.52 P-Value=0.0001	6±0.023 C.V(%)=0.66 P-Value=0.0001	2206±12.71 C.V(%)=0.99 P-Value=0.0001	462±2.890 C.V(%)=1.08 P-Value=0.0001	↑	↓	↓	N	↓	↓
B-6	6±0.028 C.V(%)=0.83 P-Value=0.0000	120±1.156 C.V(%)=1.66 P-Value=0.0001	38±0.115 C.V(%)=0.52 P-Value=0.0001	4±0.005 C.V(%)=0.25 P-Value=0.0001	1974±9.248 C.V(%)=0.81 P-Value=0.0001	430±4.046 C.V(%)=1.62 P-Value=0.0001	↓	↑	↓	↓	↓	↓
B-7	8±0.057 C.V(%)=1.25 P-Value=0.0000	52±0.173 C.V(%)=0.57 P-Value=0.0001	38±0.144 C.V(%)=0.65 P-Value=0.0001	6±0.017 C.V(%)=0.50 P-Value=0.0001	2218±6.936 C.V(%)=0.54 P-Value=0.0001	536±3.468 C.V(%)=1.11 P-Value=0.0001	N	↓	↓	N	↓	↓
B-8	10±0.144 C.V(%)=2.50 P-Value=0.0002	64±0.144 C.V(%)=0.39 P-Value=0.0001	36±0.104 C.V(%)=0.50 P-Value=0.0001	6±0.028 C.V(%)=0.83 P-Value=0.0001	2196±16.18 C.V(%)=1.27 P-Value=0.0001	402±4.624 C.V(%)=1.99 P-Value=0.0001	↑	↑	↓	N	↓	↓
C-9	14±0.115 C.V(%)=2.00 P-Value=0.0001	54±0.086 C.V(%)=0.27 P-Value=0.0001	44±0.202 C.V(%)=0.79 P-Value=0.0001	10±0.057 C.V(%)=1.00 P-Value=0.0001	2344±10.40 C.V(%)=0.76 P-Value=0.0001	464±5.202 C.V(%)=1.93 P-Value=0.0001	↑	↓	N	↑	↑	↓
C-10	8±0.057 C.V(%)=1.50 P-Value=0.0001	116±1.156 C.V(%)=1.72 P-Value=0.0001	42±0.260 C.V(%)=1.07 P-Value=0.0001	8±0.046 C.V(%)=1.00 P-Value=0.0001	2364±10.40 C.V(%)=0.76 P-Value=0.0001	426±2.890 C.V(%)=1.17 P-Value=0.0001	N	↑	↓	↑	↑	↓
C-11	10±0.086 C.V(%)=1.50 P-Value=0.0001	46±0.231 C.V(%)=0.86 P-Value=0.0001	40±0.242 C.V(%)=1.05 P-Value=0.0001	8±0.011 C.V(%)=0.25 P-Value=0.0001	2368±16.18 C.V(%)=1.18 P-Value=0.0001	540±4.046 C.V(%)=1.29 P-Value=0.0001	↑	↓	↓	↑	↑	↓
C-12	8±0.057 C.V(%)=1.25 P-Value=0.0001	58±0.144 C.V(%)=0.43 P-Value=0.0001	44±0.231 C.V(%)=0.90 P-Value=0.0001	6±0.034 C.V(%)=1.00 P-Value=0.0001	2264±5.780 C.V(%)=0.44 P-Value=0.0001	568±3.468 C.V(%)=1.05 P-Value=0.0001	C	C	C	C	C	C

N= No Increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.2a
Sample.1a. *Raphanus-sativus*. (Radish Roots)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis

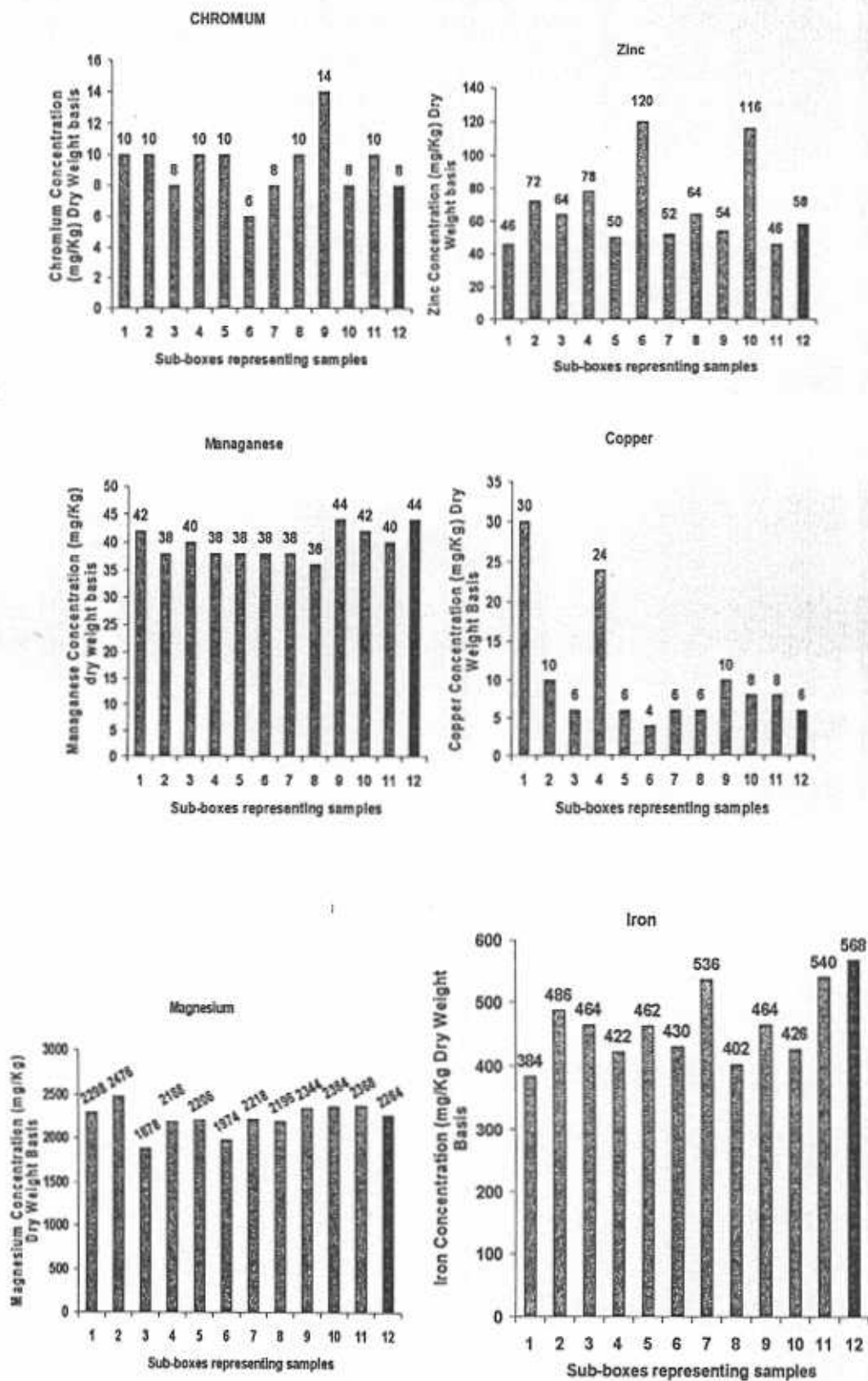
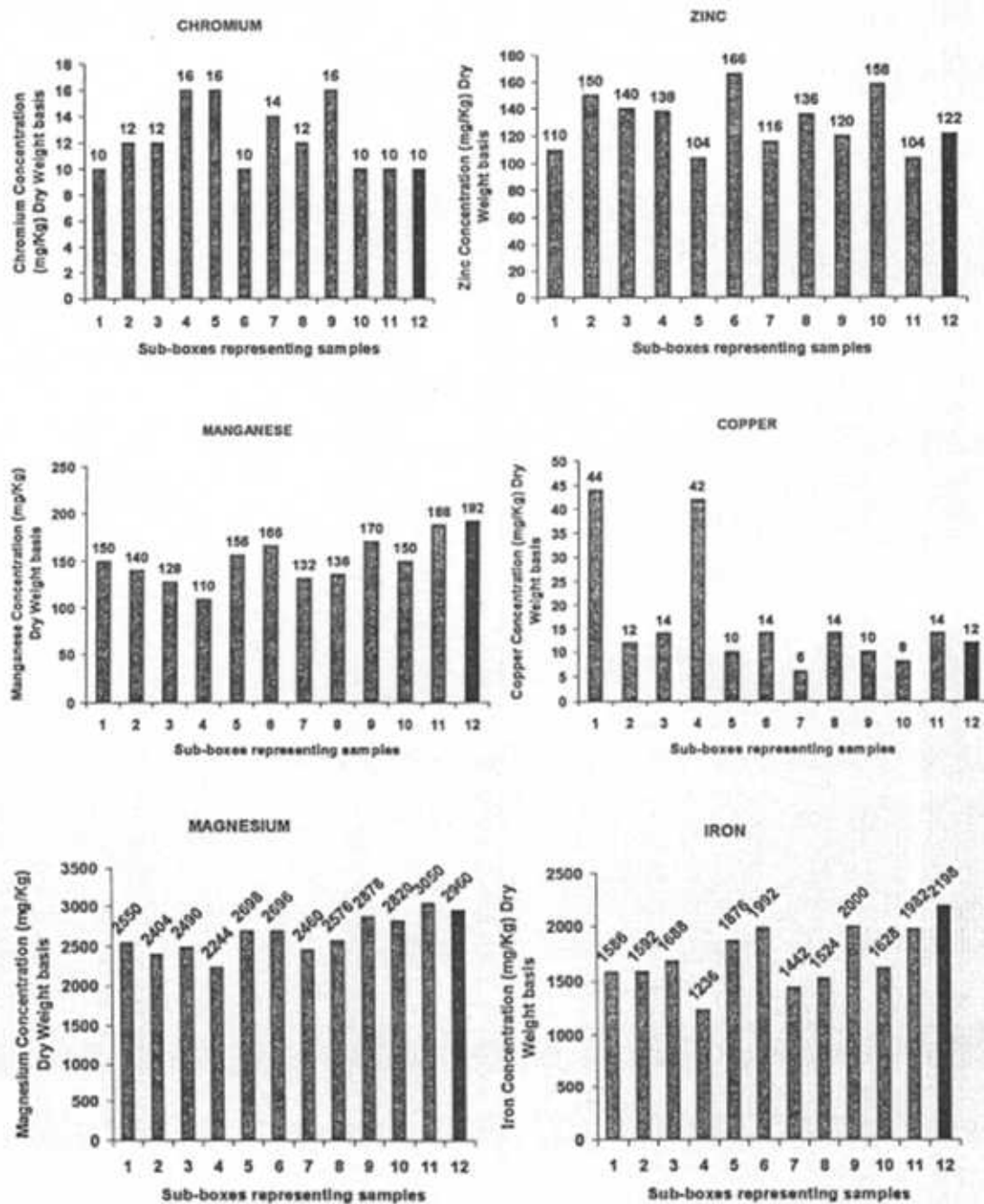


Table 3.2b *Raphanus-sativus* (Radish Leaves) Concentration of Cr, Zn, Mn, Cu, Mg and Fe (mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination Decrease(↓)/ Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	10±0.115 C.V(%)=2.00 P-Value=0.0001	110±0.867 C.V(%)=1.36 P-Value=0.0001	150±1.156 C.V(%)=1.33 P-Value=0.0001	44±0.462 C.V(%)=1.81 P-Value=0.0001	2550±6.936 C.V(%)=0.47 P-Value=0.0001	1586±13.87 C.V(%)=1.51 P-Value=0.0001	N	↓	↓	↑	↓	↓
A-2	12±0.092 C.V(%)=1.33 P-Value=0.0001	150±1.040 C.V(%)=1.20 P-Value=0.0001	140±0.867 C.V(%)=1.07 P-Value=0.0001	12±0.040 C.V(%)=0.58 P-Value=0.0001	2404±16.18 C.V(%)=1.16 P-Value=0.0001	1592±30.05 C.V(%)=3.26 P-Value=0.0004	↑	↑	↓	N	↑	↑
A-3	12±0.080 C.V(%)=1.16 P-Value=0.0001	140±0.693 C.V(%)=0.85 P-Value=0.0001	128±0.751 C.V(%)=1.01 P-Value=0.0001	14±0.231 C.V(%)=2.85 P-Value=0.0003	2490±8.092 C.V(%)=0.56 P-Value=0.0001	1688±25.43 C.V(%)=2.60 P-Value=0.0002	↑	↑	↓	↑	↑	↓
A-4	16±0.231 C.V(%)=2.50 P-Value=0.0002	138±1.156 C.V(%)=1.44 P-Value=0.0001	110±1.156 C.V(%)=1.81 P-Value=0.0001	42±0.404 C.V(%)=1.66 P-Value=0.0001	2244±8.092 C.V(%)=0.62 P-Value=0.0001	1236±8.092 C.V(%)=1.13 P-Value=0.0001	↑	↑	↓	↑	↓	↓
B-5	16±0.219 C.V(%)=2.37 P-Value=0.0002	104±0.520 C.V(%)=0.86 P-Value=0.0001	156±1.445 C.V(%)=1.60 P-Value=0.0001	10±0.115 C.V(%)=2.00 P-Value=0.0001	2698±10.40 C.V(%)=0.66 P-Value=0.0001	1876±5.780 C.V(%)=0.53 P-Value=0.0001	↑	↓	↓	↓	↓	↓
B-6	10±0.104 C.V(%)=1.80 P-Value=0.0001	166±0.809 C.V(%)=0.84 P-Value=0.0001	166±1.734 C.V(%)=1.80 P-Value=0.0001	14±0.144 C.V(%)=1.78 P-Value=0.0001	2696±8.092 C.V(%)=0.51 P-Value=0.0001	1992±10.40 C.V(%)=0.904 P-Value=0.0001	N	↑	↓	↑	↓	↓
B-7	14±0.173 C.V(%)=2.14 P-Value=0.0001	116±1.156 C.V(%)=1.72 P-Value=0.0001	132±0.867 C.V(%)=1.13 P-Value=0.0001	6±0.023 C.V(%)=0.66 P-Value=0.0001	2460±30.05 C.V(%)=2.11 P-Value=0.0001	1442±6.936 C.V(%)=0.83 P-Value=0.0001	↑	↓	↓	↓	↓	↓
B-8	12±0.115 C.V(%)=1.66 P-Value=0.0001	136±0.924 C.V(%)=1.17 P-Value=0.0001	136±0.578 C.V(%)=0.73 P-Value=0.0001	14±0.184 C.V(%)=2.28 P-Value=0.0002	2576±6.936 C.V(%)=0.46 P-Value=0.0001	1524±8.092 C.V(%)=0.91 P-Value=0.0001	↑	↑	↓	↑	↓	↓
C-9	16±0.231 C.V(%)=2.50 P-Value=0.0002	120±1.156 C.V(%)=1.66 P-Value=0.0001	170±1.560 C.V(%)=1.58 P-Value=0.0001	10±0.109 C.V(%)=1.90 P-Value=0.0001	2878±12.71 C.V(%)=0.76 P-Value=0.0001	2000±9.248 C.V(%)=0.80 P-Value=0.0001	↑	↓	↓	↓	↓	↑
C-10	10±0.121 C.V(%)=2.10 P-Value=0.0001	158±0.578 C.V(%)=0.63 P-Value=0.0001	150±1.156 C.V(%)=1.33 P-Value=0.0001	8±0.034 C.V(%)=0.75 P-Value=0.0001	2820±15.02 C.V(%)=0.92 P-Value=0.0001	1628±21.96 C.V(%)=2.33 P-Value=0.0002	N	↑	↓	↓	↓	↓
C-11	10±0.104 C.V(%)=1.80 P-Value=0.0001	104±0.578 C.V(%)=0.96 P-Value=0.0001	188±0.809 C.V(%)=0.74 P-Value=0.0001	14±0.173 C.V(%)=2.14 P-Value=0.0002	3050±9.248 C.V(%)=0.52 P-Value=0.0001	1982±18.49 C.V(%)=1.61 P-Value=0.0001	N	↓	↓	↑	↑	↓
C-12	10±0.115 C.V(%)=2.00 P-Value=0.0001	122±1.156 C.V(%)=1.63 P-Value=0.0001	192±1.156 C.V(%)=1.04 P-Value=0.0001	12±0.046 C.V(%)=0.66 P-Value=0.0001	2960±33.52 C.V(%)=1.95 P-Value=0.0001	2198±9.248 C.V(%)=0.72 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.2b
Sample.1b. *Raphanus-sativus*. (Radish Leaves)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



2. *Brassica-rapa* (Turnip Roots and Leaves)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples of roots observed was (6 ± 0.023 , 78 ± 0.231 , 50 ± 0.404 , 8 ± 0.034 , 3054 ± 12.71 and 620 ± 2.312 with a maximum concentration of Cr, Zn, Cu and Fe 24 ± 0.231 , 146 ± 0.693 , 12 ± 0.104 and 808 ± 2.312) mg/Kg on dry weight basis in sub-boxes (B-5), (A-2), (A-1) and (C-9) respectively. The concentration of Mn and Mg did not exceed in any sub-box with respect to control sample. The total increase of Cr, Zn, Cu and Fe recorded was (18, 68, 4 and 188) mg/Kg dry weight basis. While in sub-boxes (C-11), (B-5), (A-2), (A-2) and (A-4) minimum concentration of Zn, Mn, Cu, Mg and Fe (62 ± 0.346 , 32 ± 0.173 , 4 ± 0.005 , 2002 ± 15.02 and 504 ± 2.312 with total decrease of 16, 18, 4, 1052 and 116) mg/Kg dry weight basis was observed (Table 3.3a and Figure 3.3a).

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples of leaves observed was (8 ± 0.028 , 88 ± 0.578 , 142 ± 0.867 , 12 ± 0.092 , 3368 ± 13.87 and 1854 ± 6.936 with a maximum concentration of Cr, Zn, Cu, Mg and Fe (24 ± 0.231 , 152 ± 0.924 , 18 ± 0.115 , 3432 ± 9.248 and 1926 ± 12.71) mg/Kg on dry weight basis in sub-boxes (C-9), (B-6), (A-4), (B-7) and (B-5) respectively. The concentration of Mn in leaves did not exceed in any sub-box with respect to control sample. The total increase of Cr, Zn, Cu, Mg and Fe recorded was (16, 64, 6, 64 and 72) mg/Kg dry weight basis. While in sub-boxes (C-9), (B-6), (A-3, B-7), (A-2) and (A-2) minimum concentration of Zn, Mn, Cu, Mg and Fe (74 ± 0.462 , 106 ± 0.520 , (8 ± 0.028 , 8 ± 0.023), 2746 ± 6.936 and 1138 ± 15.02 with total decrease of 14, 36, 4, 622 and 716) mg/Kg dry weight basis was observed (Table 3.3b and Figure 3.3b).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application.

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). The chromium concentration was enhanced in all the sub-boxes with only exception of sub-box (A-3) of turnip roots. Marked increase was found in sub-box (B-5) at 1st stage while in sub-box (C-9) at 2nd stage of supplementation in roots and leaves respectively.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Increase was observed in all sub-boxes both in turnip roots and leaves supplemented with said combinations. Marked increase was occurred in sub-box (A-2) of turnip roots and (B-6) of turnip leaves at 1st and 2nd stages of supplementation respectively.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Decrease was observed in magnesium concentration in all sub-boxes supplemented either in combination or individually except with an increase in sub-box (B-7) and (C-11) of turnip leaves at both 1st and 2nd stages of supplementation.

Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. The copper concentration was increased in sub-boxes (A-1, A-4) and manganese decreased in sub-boxes (A-2, A-4) of turnip roots and leaves, while iron concentration was increased in sub-boxes (A-3, A-4) and increased in (A-3, A-4) of both turnip roots and leaves respectively.

Table 3.3a *Brassica-rapa* (Turnip Roots) Concentration of Cr, Zn, Mn, Cu, Mg and Fe (mg/Kg) Dry Weight Basis and Effect on their Uptake when supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination Decrease(↓) Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	10±0.057 C.V(%)=1.00 P-Value=0.0001	70±0.578 C.V(%)=1.42 P-Value=0.0001	46±0.346 C.V(%)=1.30 P-Value=0.0001	12±0.104 C.V(%)=1.50 P-Value=0.0001	2252±10.40 C.V(%)=0.79 P-Value=0.0001	640±3.468 C.V(%)=0.93 P-Value=0.0001	↑	↓	↓	↑	↓	↑
A-2	8±0.046 C.V(%)=1.00 P-Value=0.0001	146±0.693 C.V(%)=0.82 P-Value=0.0001	34±0.144 C.V(%)=0.73 P-Value=0.0001	4±0.005 C.V(%)=0.25 P-Value=0.0001	2002±15.02 C.V(%)=1.29 P-Value=0.0001	420±4.624 C.V(%)=1.90 P-Value=0.0001	↑	↑	↓	↓	↑	↓
A-3	6±0.011 C.V(%)=0.33 P-Value=0.0001	142±1.156 C.V(%)=1.40 P-Value=0.0001	44±0.260 C.V(%)=1.02 P-Value=0.0001	8±0.023 C.V(%)=0.50 P-Value=0.0001	2170±21.96 C.V(%)=1.75 P-Value=0.0001	714±3.468 C.V(%)=0.84 P-Value=0.0001	N	↑	↓	N	↓	↑
A-4	10±0.069 C.V(%)=1.20 P-Value=0.0001	116±0.924 C.V(%)=1.37 P-Value=0.0001	40±0.231 C.V(%)=1.00 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	2276±15.02 C.V(%)=1.14 P-Value=0.0001	504±2.312 C.V(%)=0.79 P-Value=0.0001	↑	↑	↓	↑	↓	↓
B-5	24±0.231 C.V(%)=1.66 P-Value=0.0001	74±0.520 C.V(%)=1.21 P-Value=0.0001	32±0.173 C.V(%)=0.93 P-Value=0.0001	6±0.023 C.V(%)=0.66 P-Value=0.0001	2332±6.936 C.V(%)=0.51 P-Value=0.0001	640±1.734 C.V(%)=0.46 P-Value=0.0001	↑	↑	↓	↓	↓	↑
B-6	6±0.017 C.V(%)=0.50 P-Value=0.0001	108±0.867 C.V(%)=1.38 P-Value=0.0001	34±0.144 C.V(%)=0.73 P-Value=0.0001	6±0.023 C.V(%)=0.66 P-Value=0.0001	2108±6.936 C.V(%)=0.56 P-Value=0.0001	534±1.156 C.V(%)=0.37 P-Value=0.0001	N	↑	↓	↓	↓	↓
B-7	10±0.075 C.V(%)=1.30 P-Value=0.0001	64±0.289 C.V(%)=0.78 P-Value=0.0001	36±0.289 C.V(%)=1.38 P-Value=0.0001	8±0.040 C.V(%)=0.87 P-Value=0.0001	2488±5.780 C.V(%)=0.40 P-Value=0.0001	542±2.312 C.V(%)=0.73 P-Value=0.0001	↑	↓	↓	N	↓	↓
B-8	20±0.115 C.V(%)=1.00 P-Value=0.0001	88±0.462 C.V(%)=0.90 P-Value=0.0001	42±0.346 C.V(%)=1.42 P-Value=0.0001	8±0.028 C.V(%)=0.62 P-Value=0.0001	2628±26.58 C.V(%)=1.75 P-Value=0.0001	640±1.156 C.V(%)=0.31 P-Value=0.0001	↑	↑	↓	N	↓	↑
C-9	20±0.173 C.V(%)=1.50 P-Value=0.0001	76±0.404 C.V(%)=0.92 P-Value=0.0001	46±0.289 C.V(%)=1.08 P-Value=0.0001	6±0.011 C.V(%)=0.33 P-Value=0.0001	2718±13.87 C.V(%)=0.88 P-Value=0.0001	808±2.312 C.V(%)=0.49 P-Value=0.0001	↑	↓	↓	↓	↓	↑
C-10	8±0.034 C.V(%)=0.75 P-Value=0.0001	102±1.156 C.V(%)=1.96 P-Value=0.0001	40±0.202 C.V(%)=0.87 P-Value=0.0001	8±0.028 C.V(%)=0.62 P-Value=0.0001	2608±9.248 C.V(%)=0.61 P-Value=0.0001	636±3.468 C.V(%)=0.94 P-Value=0.0001	↑	↑	↓	N	↓	↑
C-11	8±0.052 C.V(%)=1.12 P-Value=0.0001	62±0.346 C.V(%)=0.96 P-Value=0.0001	38±0.173 C.V(%)=0.78 P-Value=0.0001	6±0.017 C.V(%)=0.50 P-Value=0.0001	2490±11.56 C.V(%)=0.80 P-Value=0.0001	484±1.156 C.V(%)=0.41 P-Value=0.0001	↑	↓	↓	↓	↓	↓
C-12	6±0.023 C.V(%)=0.66 P-Value=0.0001	78±0.231 C.V(%)=0.51 P-Value=0.0001	50±0.404 C.V(%)=1.40 P-Value=0.0001	8±0.034 C.V(%)=0.75 P-Value=0.0001	3054±12.71 C.V(%)=0.72 P-Value=0.0001	620±2.312 C.V(%)=0.64 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.3a
Sample. 2a. *Brassica-Rapa* (Turnip Roots)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis

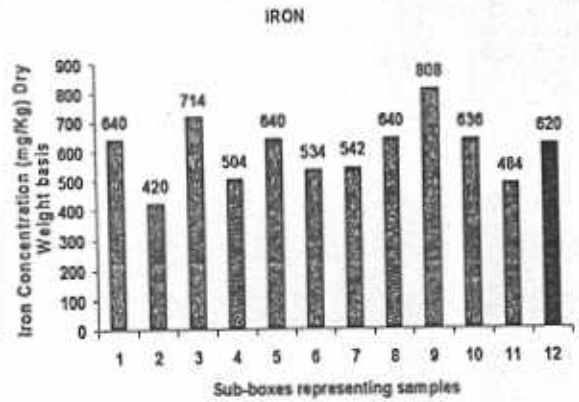
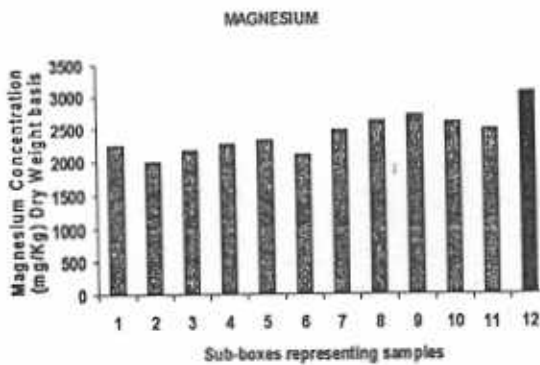
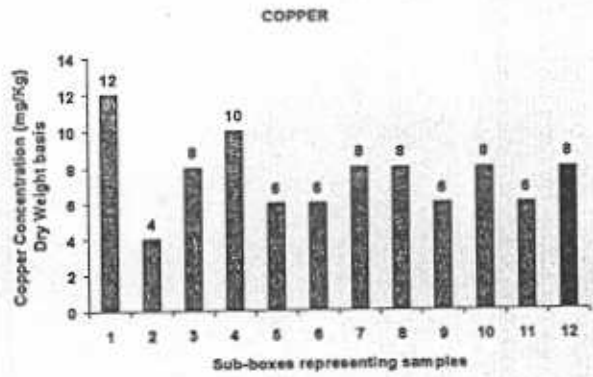
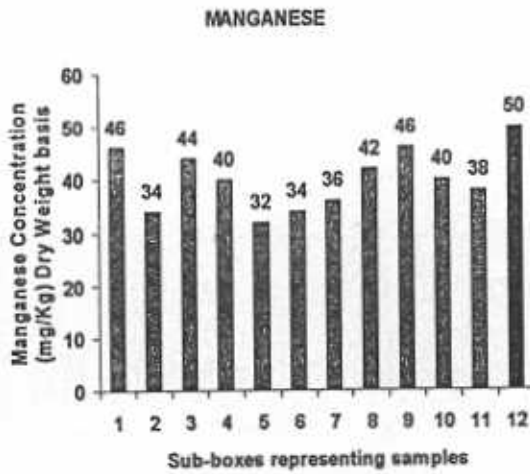
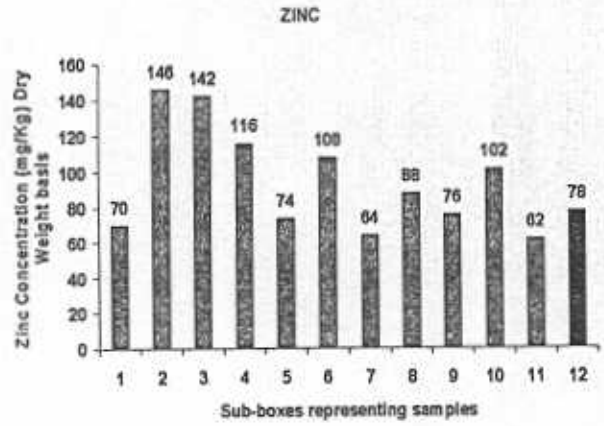
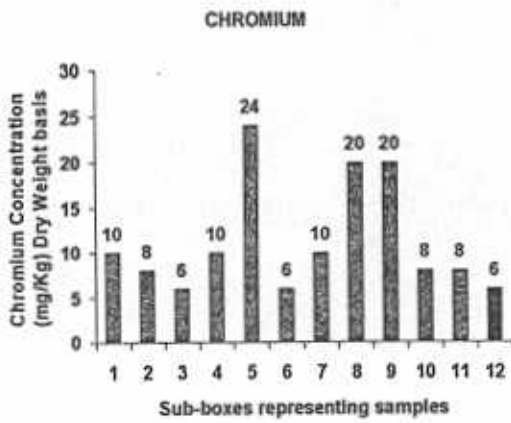
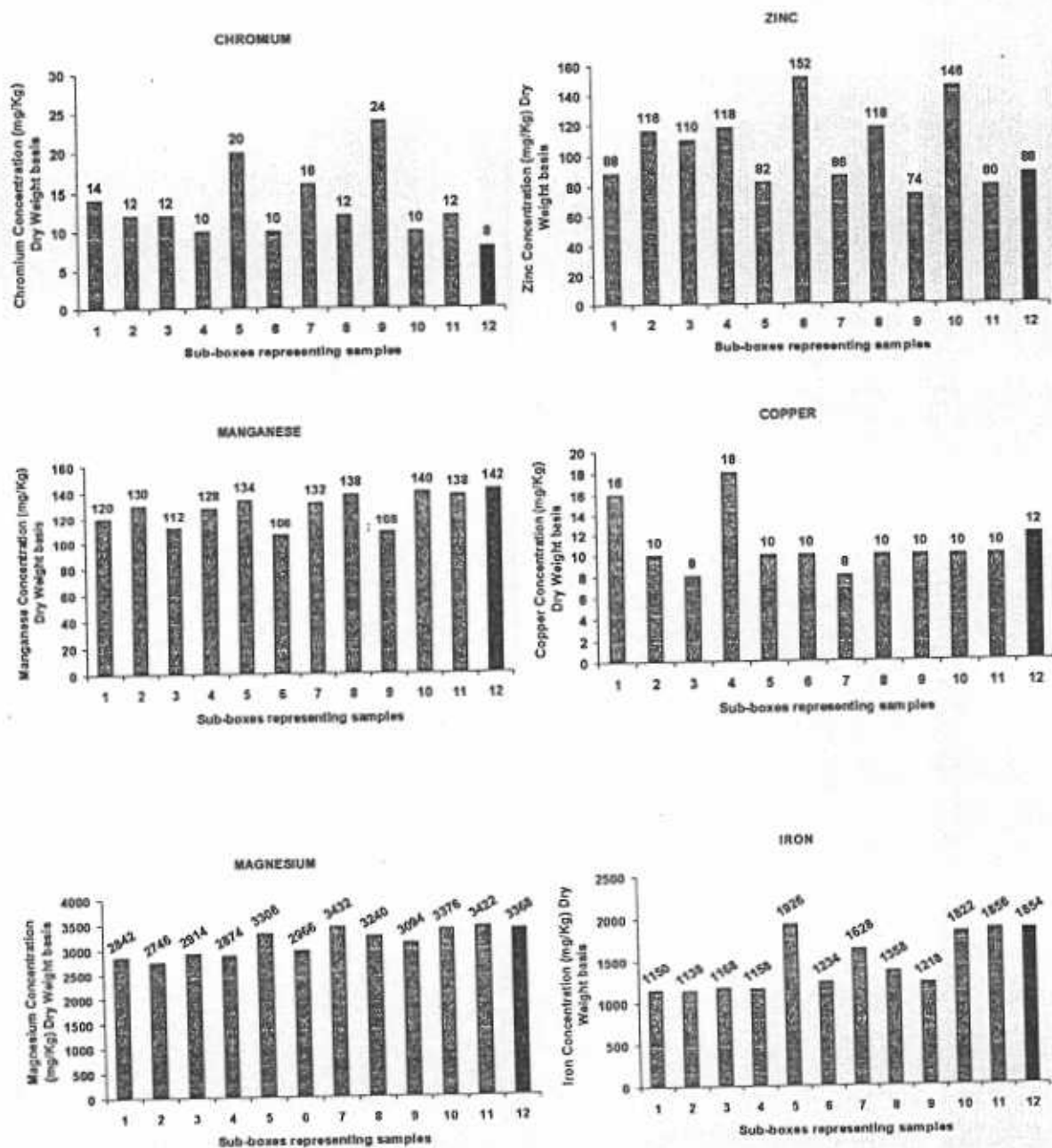


Table 3.3 b *Brassica-rapa* (Turnip Leaves) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	14±0.115 C.V(%)=1.42 P-Value=0.0001	88±0.578 C.V(%)=1.13 P-Value=0.0001	120±0.867 C.V(%)=1.25 P-Value=0.0001	16±0.092 C.V(%)=1.00 P-Value=0.0001	2842±10.40 C.V(%)=0.63 P-Value=0.0001	1150±10.40 C.V(%)=1.56 P-Value=0.0001	↑	N	↓	↑	↑	↓
A-2	12±0.104 C.V(%)=1.50 P-Value=0.0001	116±0.809 C.V(%)=1.20 P-Value=0.0001	130±0.693 C.V(%)=0.92 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	2746±6.936 C.V(%)=0.43 P-Value=0.0001	1138±15.02 C.V(%)=2.28 P-Value=0.0002	↑	↑	↓	↓	↓	↓
A-3	12±0.115 C.V(%)=1.66 P-Value=0.0001	110±0.924 C.V(%)=1.45 P-Value=0.0001	112±1.156 C.V(%)=1.78 P-Value=0.0001	8±0.028 C.V(%)=0.62 P-Value=0.0001	2914±18.49 C.V(%)=1.09 P-Value=0.0001	1168±5.780 C.V(%)=0.85 P-Value=0.0001	↑	↑	↓	↓	↓	↓
A-4	10±0.052 C.V(%)=0.90 P-Value=0.0001	118±1.156 C.V(%)=1.69 P-Value=0.0001	128±1.040 C.V(%)=1.40 P-Value=0.0001	18±0.115 C.V(%)=1.11 P-Value=0.0001	2874±15.02 C.V(%)=0.90 P-Value=0.0001	1158±6.936 C.V(%)=1.03 P-Value=0.0001	↑	↑	↓	↑	↓	↓
B-5	20±0.202 C.V(%)=1.75 P-Value=0.0001	82±0.520 C.V(%)=1.09 P-Value=0.0001	134±0.924 C.V(%)=1.19 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	3306±12.71 C.V(%)=0.66 P-Value=0.0001	1926±12.71 C.V(%)=1.14 P-Value=0.0001	↑	↓	↓	↓	↓	↑
B-6	10±0.046 C.V(%)=0.80 P-Value=0.0001	152±0.924 C.V(%)=1.05 P-Value=0.0001	106±0.520 C.V(%)=0.84 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	2966±12.71 C.V(%)=0.74 P-Value=0.0001	1234±9.248 C.V(%)=1.29 P-Value=0.0001	↑	↑	↓	↓	↓	↓
B-7	16±0.104 C.V(%)=1.12 P-Value=0.0001	86±0.578 C.V(%)=1.16 P-Value=0.0001	132±0.578 C.V(%)=0.75 P-Value=0.0001	8±0.023 C.V(%)=0.50 P-Value=0.0001	3432±9.248 C.V(%)=0.46 P-Value=0.0001	1628±6.936 C.V(%)=0.73 P-Value=0.0001	↑	↓	↓	↓	↓	↓
B-8	12±0.092 C.V(%)=1.33 P-Value=0.0001	118±1.040 C.V(%)=1.52 P-Value=0.0001	138±0.867 C.V(%)=1.08 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	3240±10.40 C.V(%)=0.55 P-Value=0.0001	1358±20.80 C.V(%)=2.65 P-Value=0.0002	↑	↑	↓	↓	↓	↓
C-9	24±0.231 C.V(%)=1.66 P-Value=0.0001	74±0.462 C.V(%)=1.08 P-Value=0.0001	108±0.578 C.V(%)=0.92 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	3094±9.248 C.V(%)=0.51 P-Value=0.0001	1218±10.40 C.V(%)=1.47 P-Value=0.0001	↑	↓	↓	↓	↓	↓
C-10	10±0.057 C.V(%)=1.00 P-Value=0.0001	146±0.809 C.V(%)=0.95 P-Value=0.0001	140±0.693 C.V(%)=0.85 P-Value=0.0001	10±0.028 C.V(%)=0.50 P-Value=0.0001	3376±18.49 C.V(%)=0.94 P-Value=0.0001	1822±10.40 C.V(%)=0.98 P-Value=0.0001	↑	↑	↓	↓	↓	↓
C-11	12±0.098 C.V(%)=1.41 P-Value=0.0001	80±0.462 C.V(%)=1.00 P-Value=0.0001	138±0.924 C.V(%)=1.15 P-Value=0.0001	10±0.034 C.V(%)=0.60 P-Value=0.0001	3422±18.49 C.V(%)=0.93 P-Value=0.0001	1856±16.18 C.V(%)=1.50 P-Value=0.0001	↑	↓	↓	↓	↓	↓
C-12	8±0.028 C.V(%)=0.62 P-Value=0.0001	88±0.578 C.V(%)=1.13 P-Value=0.0001	142±0.867 C.V(%)=1.05 P-Value=0.0001	12±0.092 C.V(%)=1.33 P-Value=0.0001	3368±13.87 C.V(%)=0.71 P-Value=0.0001	1854±6.936 C.V(%)=0.64 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.3b
Sample. 2b. *Brassica-Rapa* (Turnip Roots)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



3. *Spinacea-oleracea* (Spinach) winter

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (22 ± 0.173 , 130 ± 0.867 , 164 ± 1.156 , 18 ± 0.057 , 14740 ± 9.248 and 3124 ± 10.40 with a maximum concentration of Cr, Zn, Mn, Mg and Fe 42 ± 0.115 , 196 ± 1.734 , 196 ± 1.156 , 16460 ± 9.248 and 3498 ± 8.092) mg/Kg on dry weight basis in sub-boxes (B-5), (A-4), (A-4), (C-9) and (A-4) respectively. Only concentration of Cu did not increase or decrease with respect to control sample. The total increase of Cr, Zn, Mn, Mg and Fe recorded was (20, 66, 32, 1720 and 374) mg/Kg dry weight basis. While in sub-boxes (B-6), (B-7), (B-7), (B-7), (B-7) and (C-10) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (10 ± 0.040 , 110 ± 0.924 , 110 ± 0.462 , 8 ± 0.046 , 9800 ± 11.56 and 2074 ± 6.936 with total decrease of 12, 20, 54, 10, 4940 and 1050) mg/Kg dry weight basis was observed (Table 3.4 and Figure 3.4).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). The concentration was increased in sub-boxes (A-1, B-8, B-5, B-8, C-9) with a marked increase in sub-box (A-5) at 1st stage of supplementation and decreased in sub-boxes (A-2, A-3, A-4). Chromium content was decreased where magnesium and zinc was applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Increase was observed in all cases. Zinc content decreased, where chromium and zinc were applied alone. Marked increase was observed in sub-box (A-4).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Decrease was occurred in sub-boxes (A-1, B-8) and (B-7), while increased concentration in sub-boxes (A-4) and (C-11). Marked increase was recorded in sub-box (C-9) in case of chromium supplementation (Ind-9).

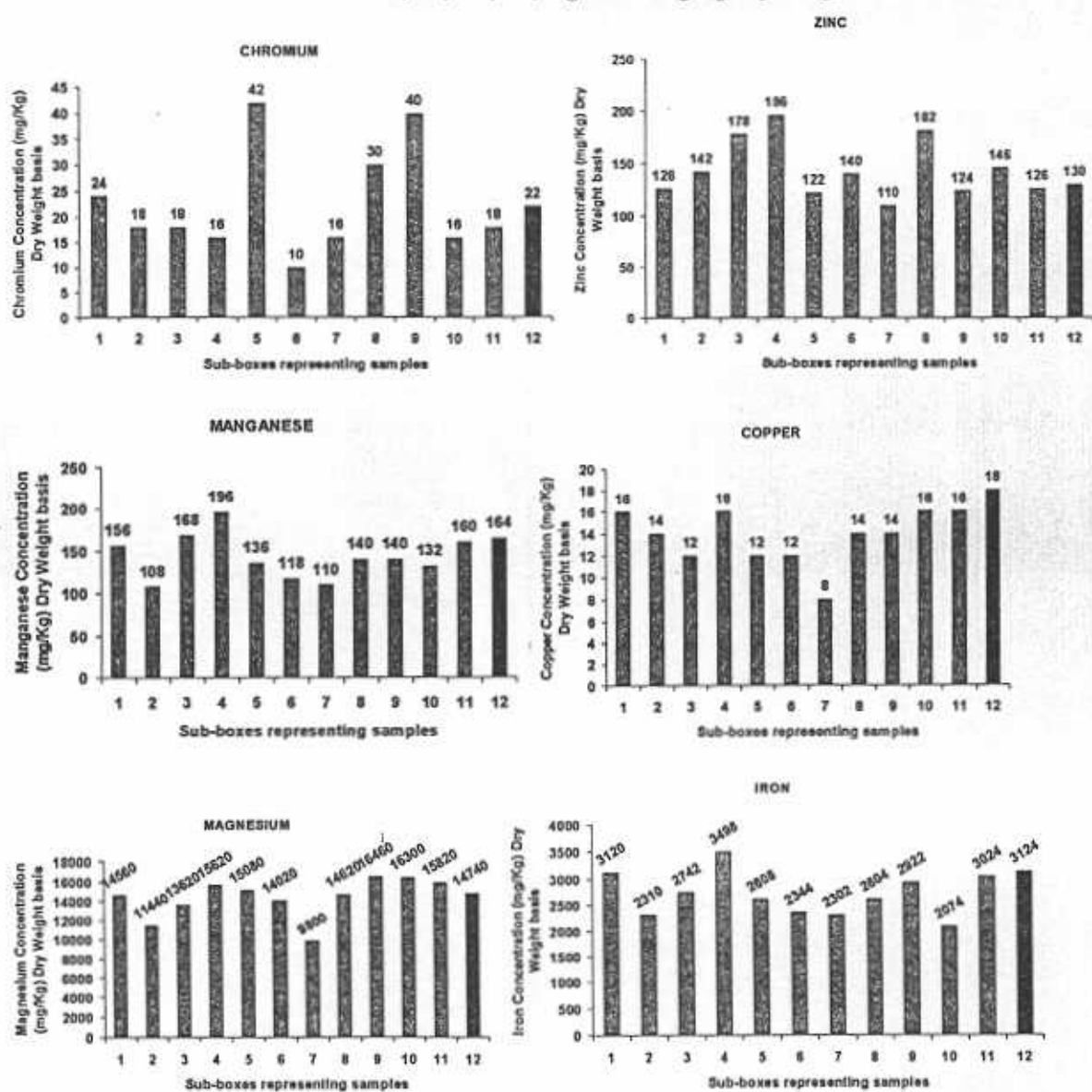
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Copper content was decreased in both cases, while manganese and iron content was increased in sub-box (A-4) and decreased in sub-boxes (A-2), (A-3) respectively.

Table 3.4 *Spinacea-oleracea*(Spinach) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	24±0.202 C.V(%)=1.45 P-Value=0.0001	126±0.578 C.V(%)=0.79 P-Value=0.0001	156±0.809 C.V(%)=0.89 P-Value=0.0001	16±0.104 C.V(%)=1.12 P-Value=0.0001	14560±30.05 C.V(%)=0.35 P-Value=0.0001	3120±10.40 C.V(%)=0.57 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-2	18±0.104 C.V(%)=1.00 P-Value=0.0001	142±1.156 C.V(%)=1.40 P-Value=0.0001	108±0.693 C.V(%)=1.11 P-Value=0.0001	14±0.115 C.V(%)=1.42 P-Value=0.0001	11440±18.49 C.V(%)=0.27 P-Value=0.0001	2310±8.092 C.V(%)=0.60 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-3	18±0.092 C.V(%)=0.88 P-Value=0.0001	178±2.312 C.V(%)=2.24 P-Value=0.0002	168±0.924 C.V(%)=0.95 P-Value=0.0001	12±0.092 C.V(%)=1.33 P-Value=0.0001	13620±25.43 C.V(%)=0.32 P-Value=0.0001	2742±16.18 C.V(%)=1.02 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-4	16±0.092 C.V(%)=1.00 P-Value=0.0001	196±1.734 C.V(%)=1.53 P-Value=0.0001	196±1.156 C.V(%)=1.02 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	15620±8.092 C.V(%)=0.08 P-Value=0.0001	3498±8.092 C.V(%)=0.40 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	42±0.115 C.V(%)=0.47 P-Value=0.0001	122±1.040 C.V(%)=1.47 P-Value=0.0001	136±0.809 C.V(%)=1.02 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	15080±5.780 C.V(%)=0.06 P-Value=0.0001	2608±10.40 C.V(%)=0.69 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-6	10±0.040 C.V(%)=0.70 P-Value=0.0001	140±0.924 C.V(%)=1.14 P-Value=0.0001	118±0.924 C.V(%)=1.35 P-Value=0.0001	12±0.092 C.V(%)=1.33 P-Value=0.0001	14020±10.40 C.V(%)=0.12 P-Value=0.0001	2344±30.05 C.V(%)=2.21 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-7	16±0.115 C.V(%)=1.25 P-Value=0.0001	110±0.924 C.V(%)=1.45 P-Value=0.0001	110±0.462 C.V(%)=0.72 P-Value=0.0001	8±0.046 C.V(%)=1.00 P-Value=0.0001	9800±11.56 C.V(%)=0.20 P-Value=0.0001	2302±8.092 C.V(%)=0.60 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-8	30±0.202 C.V(%)=1.16 P-Value=0.0001	182±1.040 C.V(%)=0.98 P-Value=0.0001	140±0.809 C.V(%)=1.00 P-Value=0.0001	14±0.104 C.V(%)=1.28 P-Value=0.0001	14620±8.092 C.V(%)=0.09 P-Value=0.0001	2604±15.02 C.V(%)=0.99 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-9	40±0.057 C.V(%)=0.25 P-Value=0.0001	124±0.693 C.V(%)=0.96 P-Value=0.0001	140±0.924 C.V(%)=1.14 P-Value=0.0001	14±0.104 C.V(%)=1.28 P-Value=0.0001	16460±9.248 C.V(%)=0.09 P-Value=0.0001	2922±9.248 C.V(%)=0.54 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-10	16±0.092 C.V(%)=1.00 P-Value=0.0001	146±1.156 C.V(%)=1.36 P-Value=0.0001	132±0.867 C.V(%)=1.13 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	16300±21.96 C.V(%)=0.23 P-Value=0.0001	2074±6.936 C.V(%)=0.57 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-11	18±0.104 C.V(%)=1.00 P-Value=0.0001	126±0.693 C.V(%)=0.95 P-Value=0.0001	160±1.156 C.V(%)=1.25 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	15820±18.49 C.V(%)=0.20 P-Value=0.0001	3024±12.71 C.V(%)=0.72 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-12	22±0.173 C.V(%)=1.36 P-Value=0.0001	130±0.867 C.V(%)=1.15 P-Value=0.0001	164±1.156 C.V(%)=1.21 P-Value=0.0001	18±0.057 C.V(%)=0.55 P-Value=0.0001	14740±9.248 C.V(%)=0.10 P-Value=0.0001	3124±10.40 C.V(%)=0.37 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.4
Sample. 3 *Spinacea-oleracea* (Spinach)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



4. *Trigonella-foenum-graceum*. (Fenugreek/ Methi)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (24 ± 0.115 , 68 ± 0.462 , 66 ± 0.323 , 14 ± 0.115 , 3008 ± 19.65 and 1572 ± 11.56 with a maximum concentration of Cr, Zn, Mn, Mg and Fe 34 ± 0.231 , 162 ± 1.156 , 76 ± 0.462 , 3264 ± 24.27 and 2092 ± 11.56) mg/Kg on dry weight basis in sub-boxes (B-5), (A-4), (A-4), (B-7) and (A-3) respectively. Only concentration of Cu did not increase or decrease with respect to control sample. The total increase of Cr, Zn, Mn, Mg and Fe recorded was (10, 94, 10, 256 and 520) mg/g dry weight basis. While in sub-boxes (B-8), (C-9), (C-9), (A-2, B-5, B-7, C-9, C-10), (A-3) and (C-9) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (12 ± 0.057 , 62 ± 0.520 , 44 ± 0.173 , ($8 \pm 0.023 - 0.040$), 1980 ± 8.092 and 1032 ± 9.248 with total decrease of 12, 6, 22, 6, 1028 and 540) mg/g dry weight basis was observed (Table 3.5 and Figure 3.5).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Decrease in chromium concentration was occurred in sub-boxes (A-1, A-4, B-8) and increase in sub-boxes (A-2, A-3) and (B-5, C-9). Marked increase was occurred in sub-box (B-5) at 1st stage of supplementation. Chromium content was decreased where magnesium and zinc was applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (A-4) at 1st stage of supplementation. Zinc content was decreased where magnesium and chromium were applied alone and also in sub-box (A-1).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes except (A-1) with marked increase in sub-box (B-7) at 1st stage of supplementation.

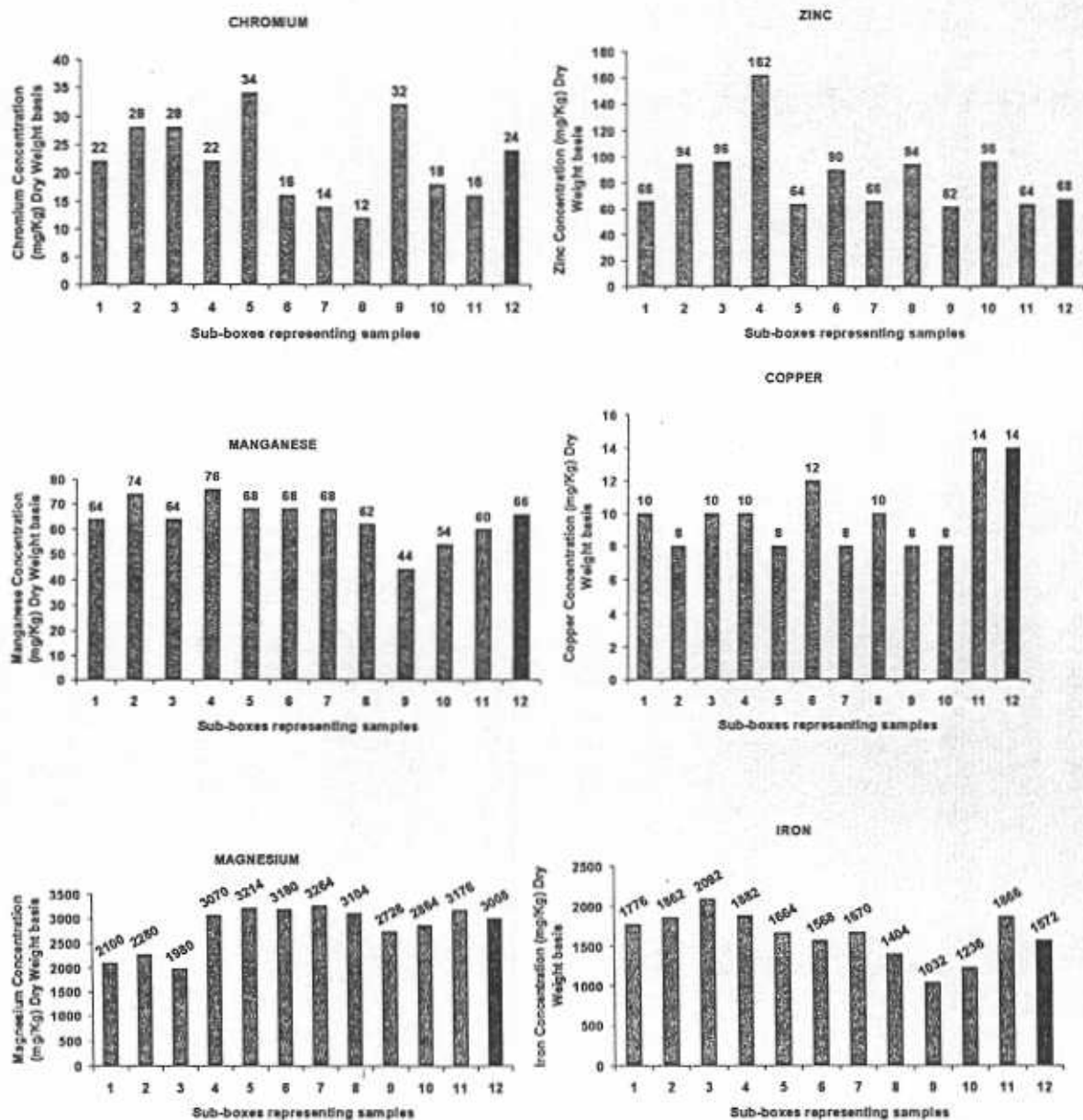
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. The copper content was decreased, while manganese and iron contents were increased in both sub-boxes with marked increase in sub-boxes (A-4) and (A-3) respectively.

Table 3.5 *Trigonella-foenum-graceum*. (Fenugreek/ Methi) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	22±0.173 C.V(%)=1.36 P-Value=0.0001	66±0.578 C.V(%)=1.51 P-Value=0.0001	64±0.578 C.V(%)=1.56 P-Value=0.0001	10±0.104 C.V(%)=1.80 P-Value=0.0001	2100±6.936 C.V(%)=0.57 P-Value=0.0001	1776±15.02 C.V(%)=1.46 P-Value=0.0001	↓	↓	↓	↓	↓	↑
A-2	28±0.144 C.V(%)=0.89 P-Value=0.0001	94±0.462 C.V(%)=0.85 P-Value=0.0001	74±0.289 C.V(%)=0.67 P-Value=0.0001	8±0.028 C.V(%)=0.62 P-Value=0.0001	2280±6.936 C.V(%)=0.52 P-Value=0.0001	1862±15.02 C.V(%)=1.39 P-Value=0.0001	↑	↑	↑	↓	↓	↑
A-3	28±0.121 C.V(%)=0.75 P-Value=0.0001	96±0.578 C.V(%)=1.04 P-Value=0.0001	64±0.462 C.V(%)=1.25 P-Value=0.0001	10±0.092 C.V(%)=1.60 P-Value=0.0001	1980±8.092 C.V(%)=0.70 P-Value=0.0001	2092±11.56 C.V(%)=0.95 P-Value=0.0001	↑	↑	↓	↓	↓	↑
A-4	22±0.202 C.V(%)=1.59 P-Value=0.0001	162±1.156 C.V(%)=1.23 P-Value=0.0001	76±0.462 C.V(%)=1.05 P-Value=0.0001	10±0.104 C.V(%)=1.80 P-Value=0.0001	3070±5.780 C.V(%)=0.32 P-Value=0.0001	1882±11.56 C.V(%)=1.06 P-Value=0.0001	↓	↑	↑	↓	↑	↑
B-5	34±0.231 C.V(%)=1.17 P-Value=0.0001	64±0.462 C.V(%)=1.25 P-Value=0.0001	68±0.346 C.V(%)=0.88 P-Value=0.0001	8±0.023 C.V(%)=0.50 P-Value=0.0001	3214±6.936 C.V(%)=0.37 P-Value=0.0001	1664±21.96 C.V(%)=2.28 P-Value=0.0001	↑	↓	↑	↓	↑	↑
B-6	16±0.098 C.V(%)=1.06 P-Value=0.0001	90±0.693 C.V(%)=1.33 P-Value=0.0001	68±0.346 C.V(%)=0.88 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	3180±6.936 C.V(%)=0.37 P-Value=0.0001	1568±13.87 C.V(%)=1.53 P-Value=0.0001	↓	↑	↑	↓	↑	↓
B-7	14±0.086 C.V(%)=1.07 P-Value=0.0001	66±0.635 C.V(%)=1.66 P-Value=0.0001	68±0.404 C.V(%)=1.02 P-Value=0.0001	8±0.040 C.V(%)=0.87 P-Value=0.0001	3264±24.27 C.V(%)=1.28 P-Value=0.0001	1670±26.58 C.V(%)=2.75 P-Value=0.0001	↓	↓	↑	↓	↑	↑
B-8	12±0.057 C.V(%)=0.83 P-Value=0.0001	94±0.520 C.V(%)=0.95 P-Value=0.0001	62±0.289 C.V(%)=0.80 P-Value=0.0001	10±0.086 C.V(%)=1.50 P-Value=0.0001	3104±11.56 C.V(%)=0.64 P-Value=0.0001	1404±11.56 C.V(%)=1.42 P-Value=0.0001	↓	↑	↓	↓	↑	↓
C-9	32±0.057 C.V(%)=0.31 P-Value=0.0001	62±0.520 C.V(%)=1.45 P-Value=0.0001	44±0.173 C.V(%)=0.68 P-Value=0.0001	8±0.040 C.V(%)=0.87 P-Value=0.0001	2726±13.87 C.V(%)=0.88 P-Value=0.0001	1032±9.248 C.V(%)=1.55 P-Value=0.0001	↑	↓	↓	↓	↓	↓
C-10	18±0.075 C.V(%)=0.72 P-Value=0.0001	96±0.462 C.V(%)=0.83 P-Value=0.0001	54±0.231 C.V(%)=0.74 P-Value=0.0001	8±0.034 C.V(%)=0.75 P-Value=0.0001	2864±15.02 C.V(%)=0.90 P-Value=0.0001	1236±12.71 C.V(%)=1.77 P-Value=0.0001	↓	↑	↓	↓	↓	↓
C-11	16±0.092 C.V(%)=1.00 P-Value=0.0001	64±0.404 C.V(%)=1.09 P-Value=0.0001	60±0.225 C.V(%)=0.65 P-Value=0.0001	14±0.104 C.V(%)=1.28 P-Value=0.0001	3176±6.936 C.V(%)=0.37 P-Value=0.0001	1866±33.52 C.V(%)=3.10 P-Value=0.0003	↓	↓	↓	N	↑	↑
C-12	24±0.115 C.V(%)=0.83 P-Value=0.0001	68±0.462 C.V(%)=1.17 P-Value=0.0001	66±0.323 C.V(%)=0.84 P-Value=0.0001	14±0.115 C.V(%)=1.42 P-Value=0.0001	3008±19.65 C.V(%)=1.13 P-Value=0.0001	1572±11.56 C.V(%)=1.27 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.5
Sample .4 *Trigonella-foenum-graceum*. (Fenugreek)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



5. *Brassica-campestris-var-sarson.*(Sarson)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (8 ± 0.040 , 92 ± 0.578 , 142 ± 0.809 , 12 ± 0.104 , 3730 ± 10.40 and 946 ± 1.156 with a maximum concentration of Cr, Zn, Mn and Mg (20 ± 0.121 , 122 ± 0.924 - 1.040 , 164 ± 0.867 and 4154 ± 9.248) mg/Kg on dry weight basis in sub-boxes (B-5), (A-3, B-6), (A-4) and (A-1) respectively. The concentration of Cu and Fe did not increase or decrease with respect to control sample. The total increase of Cr, Zn, Mn and Mg recorded was (12, 30, 22 and 424) mg/Kg dry weight basis. While in sub-boxes (B-6), (C-9), (A-1), (A-4), (B-6) and (C-9) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (6 ± 0.023 , 70 ± 0.404 , 130 ± 0.578 , 8 ± 0.023 , 3492 ± 10.40 and 638 ± 1.734 with total decrease of 2, 22, 12, 4, 238 and 308) mg/Kg dry weight basis was observed (Table 3.6 and Figure 3.6).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (B-5) at 1st stage of supplementation. Chromium concentration was increased in magnesium supplemented samples. Zinc application alone also decreased chromium concentration in sub-box (B-6) and no effect in (C-10).

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (A-3) at 1st stage of supplementation. Zinc content was decreased where magnesium and chromium were applied alone, while magnesium at later stage did not cause any change in concentration.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was decreased in all sub-boxes except in (A-1).

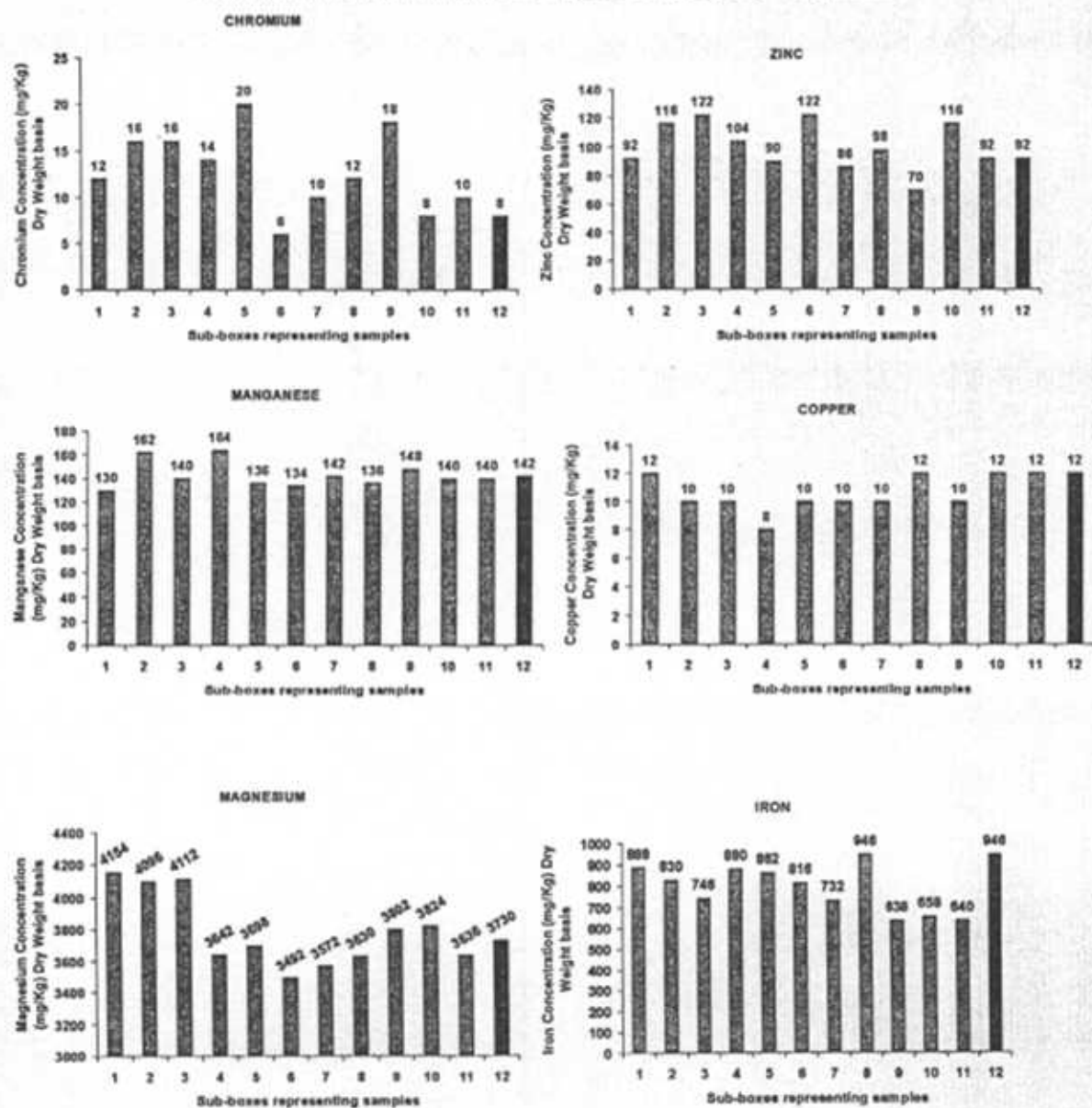
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. In sub-box (A-1) no effect while in (A-4) decrease was occurred in copper concentration. Similarly, manganese content was increased with marked increase in sub-box (A-4) and Fe content decreased in both sub-boxes (A-3, A-4).

Table 3.6 *Brassica campestris*-var-sarson. (Sarson)Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basisand Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	12±0.057 C.V(%)=0.83 P-Value=0.0001	92±0.462 C.V(%)=0.86 P-Value=0.0001	130±0.578 C.V(%)=0.76 P-Value=0.0001	12±0.092 C.V(%)=1.33 P-Value=0.0001	4154±9.248 C.V(%)=0.38 P-Value=0.0001	888±2.312 C.V(%)=0.45 P-Value=0.0001	↑	N	↑	N	↑	↑
A-2	16±0.098 C.V(%)=1.06 P-Value=0.0001	116±0.867 C.V(%)=1.29 P-Value=0.0001	162±0.578 C.V(%)=0.61 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	4096±12.71 C.V(%)=0.53 P-Value=0.0001	830±2.890 C.V(%)=0.60 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-3	16±0.092 C.V(%)=1.00 P-Value=0.0001	122±0.924 C.V(%)=1.31 P-Value=0.0001	140±0.924 C.V(%)=1.14 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	4112±6.936 C.V(%)=0.29 P-Value=0.0001	746±1.156 C.V(%)=0.26 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-4	14±0.086 C.V(%)=1.07 P-Value=0.0001	104±0.635 C.V(%)=1.05 P-Value=0.0001	164±0.867 C.V(%)=0.91 P-Value=0.0001	8±0.023 C.V(%)=0.50 P-Value=0.0001	3642±6.936 C.V(%)=0.32 P-Value=0.0001	880±2.890 C.V(%)=0.56 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	20±0.121 C.V(%)=1.05 P-Value=0.0001	90±0.693 C.V(%)=1.33 P-Value=0.0001	136±0.693 C.V(%)=0.88 P-Value=0.0001	10±0.034 C.V(%)=0.60 P-Value=0.0001	3698±15.02 C.V(%)=0.70 P-Value=0.0001	862±1.734 C.V(%)=0.34 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-6	6±0.023 C.V(%)=0.66 P-Value=0.0001	122±1.040 C.V(%)=1.47 P-Value=0.0001	134±1.156 C.V(%)=1.49 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	3492±10.40 C.V(%)=0.51 P-Value=0.0001	816±1.734 C.V(%)=0.36 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-7	10±0.034 C.V(%)=0.60 P-Value=0.0001	86±0.462 C.V(%)=0.93 P-Value=0.0001	142±0.924 C.V(%)=1.12 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	3572±5.780 C.V(%)=0.27 P-Value=0.0001	732±3.468 C.V(%)=0.81 P-Value=0.0001	↑	↑	N	↑	↑	↑
B-8	12±0.069 C.V(%)=1.00 P-Value=0.0001	98±0.520 C.V(%)=0.91 P-Value=0.0001	136±0.722 C.V(%)=0.91 P-Value=0.0001	12±0.098 C.V(%)=1.41 P-Value=0.0001	3630±20.80 C.V(%)=0.99 P-Value=0.0001	946±1.156 C.V(%)=0.21 P-Value=0.0001	↑	↑	↑	N	↑	N
C-9	18±0.075 C.V(%)=0.72 P-Value=0.0001	70±0.404 C.V(%)=1.00 P-Value=0.0001	148±1.156 C.V(%)=1.35 P-Value=0.0001	10±0.104 C.V(%)=1.80 P-Value=0.0001	3802±10.40 C.V(%)=0.47 P-Value=0.0001	638±1.734 C.V(%)=0.47 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-10	8±0.040 C.V(%)=0.87 P-Value=0.0001	116±0.809 C.V(%)=1.20 P-Value=0.0001	140±0.867 C.V(%)=1.07 P-Value=0.0001	12±0.092 C.V(%)=1.33 P-Value=0.0001	3824±10.40 C.V(%)=0.47 P-Value=0.0001	658±2.890 C.V(%)=0.75 P-Value=0.0001	N	↑	↑	N	↑	↑
C-11	10±0.028 C.V(%)=0.50 P-Value=0.0001	92±0.578 C.V(%)=1.08 P-Value=0.0001	140±0.867 C.V(%)=1.07 P-Value=0.0001	12±0.104 C.V(%)=1.50 P-Value=0.0001	3636±16.18 C.V(%)=0.77 P-Value=0.0001	640±3.468 C.V(%)=0.93 P-Value=0.0001	↑	N	↑	N	↑	↑
C-12	8±0.040 C.V(%)=0.87 P-Value=0.0001	92±0.578 C.V(%)=1.08 P-Value=0.0001	142±0.809 C.V(%)=0.98 P-Value=0.0001	12±0.104 C.V(%)=1.50 P-Value=0.0001	3730±10.40 C.V(%)=0.48 P-Value=0.0001	946±1.156 C.V(%)=0.21 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.6
Sample .5. *Brassica-campestris-var-sarson* (Sarson)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



6. *Portulaca-oleracea*. (Pot purslane)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (14 ± 0.086 , 88 ± 0.520 , 90 ± 0.578 , 14 ± 0.057 , 13660 ± 10.40 and 2864 ± 21.96 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe (54 ± 0.289 , 316 ± 2.312 , 110 ± 0.809 , 22 ± 0.173 , 23120 ± 8.092 and 3548 ± 6.936) mg/Kg on dry weight basis in sub-boxes (B-8), (B-8), (A-4), (A-1), (B-6) and (A-4) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (40, 228, 20, 8, 9460 and 684) mg/Kg dry weight basis. While in sub-boxes (B-6), (C-11), (C-11), (C-10), (A-2) and (C-11) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (12 ± 0.057 , 78 ± 0.404 , 66 ± 0.231 , 12 ± 0.057 , 12680 ± 8.092 and 1802 ± 10.40 with total decrease of 2, 10, 24, 2, 980 and 1062) mg/Kg dry weight basis was observed (Table 3.7 and Figure 3.7).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (B-8) at 1st stage of supplementation. Chromium content was increased where magnesium and zinc were applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-8) at 1st stage of supplementation. Zinc content was decreased where magnesium and chromium were applied alone, while magnesium at first stage increased zinc concentration.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was decreased in sub-boxes (A-1) and (C-11), while increased in other sub-boxes. Marked increase was occurred in sub-box (B-6) where zinc was supplemented at 1st stage of supplementation.

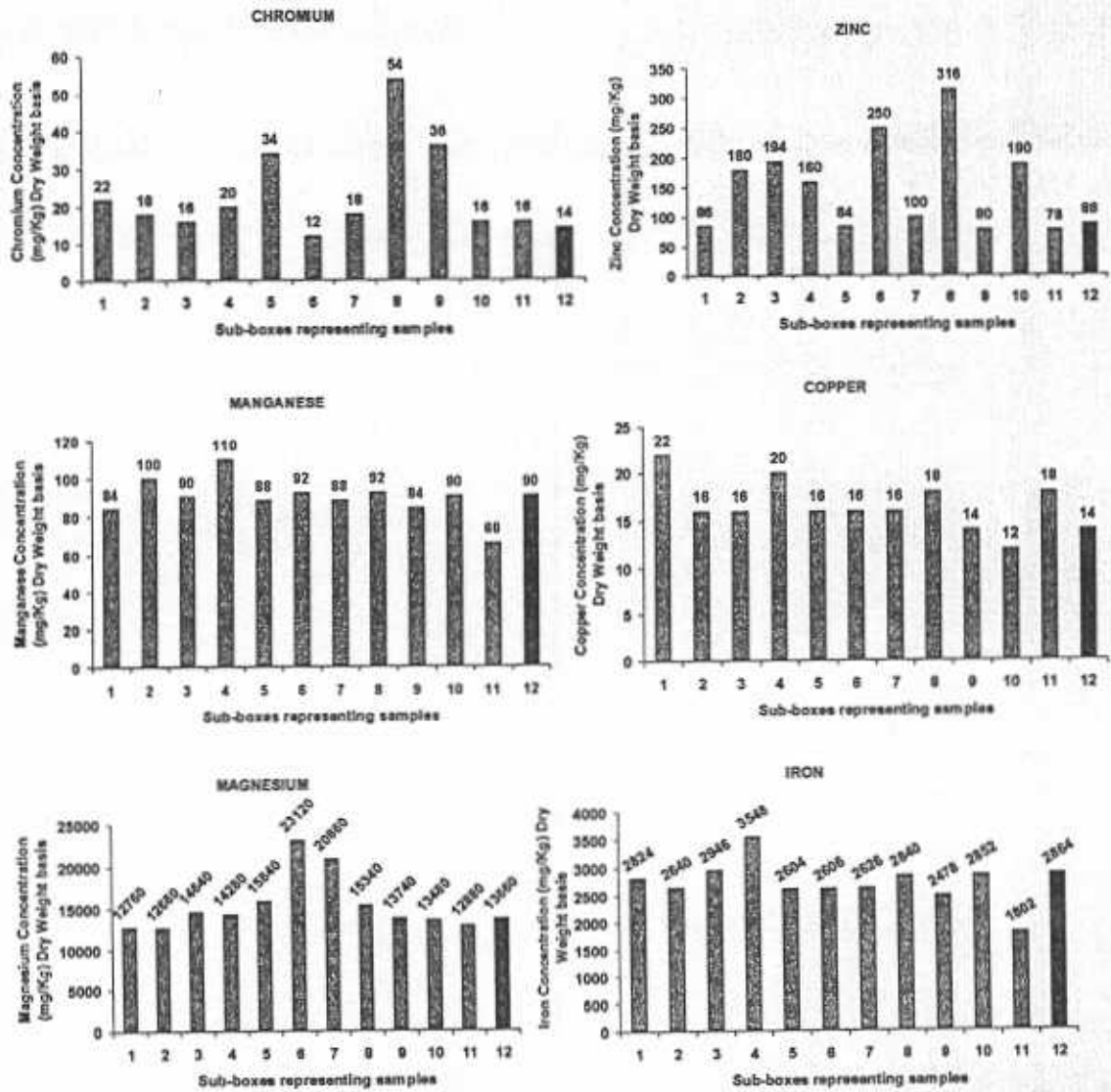
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper, manganese and iron was occurred in all sub-boxes with marked increase in (A-1), (A-4) and (A-4) respectively.

Table 3.7 *Portulaca-oleracea*. (Pot purslane) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination Decrease(↓)/ Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	22±0.202 C.V(%)=1.59 P-Value=0.0001	86±0.404 C.V(%)=0.81 P-Value=0.0001	84±0.462 C.V(%)=0.95 P-Value=0.0001	22±0.173 C.V(%)=1.36 P-Value=0.0001	12760±6.936 C.V(%)=0.09 P-Value=0.0001	2824±27.74 C.V(%)=1.69 P-Value=0.0001	↑	↓	↓	↑	↑	↑
A-2	18±0.075 C.V(%)=0.72 P-Value=0.0001	180±1.040 C.V(%)=1.00 P-Value=0.0001	100±0.693 C.V(%)=1.20 P-Value=0.0001	16±0.092 C.V(%)=1.00 P-Value=0.0001	12680±8.092 C.V(%)=0.11 P-Value=0.0001	2640±9.248 C.V(%)=0.60 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-3	16±0.086 C.V(%)=0.93 P-Value=0.0001	194±1.734 C.V(%)=1.54 P-Value=0.0001	90±0.520 C.V(%)=1.00 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	14640±8.092 C.V(%)=0.09 P-Value=0.0001	2946±15.02 C.V(%)=0.88 P-Value=0.0001	↑	↑	N	↑	↑	↑
A-4	20±0.173 C.V(%)=1.50 P-Value=0.0001	160±0.924 C.V(%)=1.00 P-Value=0.0001	110±0.809 C.V(%)=1.27 P-Value=0.0001	20±0.080 C.V(%)=0.70 P-Value=0.0001	14280±16.18 C.V(%)=0.19 P-Value=0.0001	3548±6.936 C.V(%)=0.33 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	34±0.121 C.V(%)=0.61 P-Value=0.0001	84±0.462 C.V(%)=0.95 P-Value=0.0001	88±0.404 C.V(%)=0.79 P-Value=0.0001	16±0.080 C.V(%)=0.87 P-Value=0.0001	15840±10.40 C.V(%)=0.11 P-Value=0.0001	2604±15.02 C.V(%)=0.99 P-Value=0.0001	↑	↓	↓	↑	↑	↑
B-6	12±0.057 C.V(%)=0.83 P-Value=0.0001	250±1.734 C.V(%)=1.20 P-Value=0.0001	92±0.462 C.V(%)=0.86 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	23120±8.092 C.V(%)=0.06 P-Value=0.0001	2606±17.34 C.V(%)=1.15 P-Value=0.0001	N	↑	↑	↑	↑	↑
B-7	18±0.080 C.V(%)=0.77 P-Value=0.0001	100±0.578 C.V(%)=1.00 P-Value=0.0001	88±0.346 C.V(%)=0.68 P-Value=0.0001	16±0.080 C.V(%)=0.87 P-Value=0.0001	20860±30.05 C.V(%)=0.24 P-Value=0.0001	2626±6.936 C.V(%)=0.45 P-Value=0.0001	↑	↑	↓	↑	↑	↑
B-8	54±0.289 C.V(%)=0.92 P-Value=0.0001	316±2.312 C.V(%)=1.26 P-Value=0.0001	92±0.462 C.V(%)=0.86 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	15340±15.02 C.V(%)=0.16 P-Value=0.0001	2840±12.71 C.V(%)=0.77 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-9	36±0.144 C.V(%)=0.69 P-Value=0.0001	80±0.462 C.V(%)=1.00 P-Value=0.0001	84±0.289 C.V(%)=0.59 P-Value=0.0001	14±0.057 C.V(%)=0.71 P-Value=0.0001	13740±9.248 C.V(%)=0.11 P-Value=0.0001	2478±34.68 C.V(%)=2.42 P-Value=0.0002	↑	↓	↓	N	↑	↑
C-10	16±0.098 C.V(%)=1.06 P-Value=0.0001	190±1.156 C.V(%)=1.05 P-Value=0.0001	90±0.578 C.V(%)=1.11 P-Value=0.0001	12±0.057 C.V(%)=0.83 P-Value=0.0001	13480±6.936 C.V(%)=0.08 P-Value=0.0001	2852±5.780 C.V(%)=0.35 P-Value=0.0001	↑	↑	N	↓	↑	↑
C-11	16±0.098 C.V(%)=1.06 P-Value=0.0001	78±0.404 C.V(%)=0.89 P-Value=0.0001	66±0.231 C.V(%)=0.60 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	12880±12.71 C.V(%)=0.17 P-Value=0.0001	1802±10.40 C.V(%)=0.99 P-Value=0.0001	↑	↓	↓	↑	↑	↑
C-12	14±0.086 C.V(%)=1.07 P-Value=0.0001	88±0.520 C.V(%)=1.02 P-Value=0.0001	90±0.578 C.V(%)=1.11 P-Value=0.0001	14±0.057 C.V(%)=0.71 P-Value=0.0001	13660±10.40 C.V(%)=0.13 P-Value=0.0001	2864±21.96 C.V(%)=1.32 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.7
Sample. 6. *Portulaca-oleracea*. (Pot purslane/Kolfa)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



7. *Allium-sativum.* (Garlic)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (8 ± 0.028 , 92 ± 0.520 , 20 ± 0.098 , 10 ± 0.046 , 786 ± 1.156 and 150 ± 0.693 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe (22 ± 0.173 , 120 ± 1.040 , 24 ± 0.173 , 14 ± 0.069 - 0.080 , 902 ± 1.734 and 160 ± 0.722 - 1.156) mg/Kg on dry weight basis in sub-boxes (B-5), (B-6), (A-4), (A-1,A-4,B-5,B-6),(C-10) and (A-3, B-5) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (14, 28, 4, 4, 116 and 10) mg/Kg dry weight basis. While in sub-boxes (A-1), (A-2, B-7), (A-3) and (C-9) minimum concentration of Zn, Mn, Mg and Fe (68 ± 0.578 , 18 ± 0.080 - 0.086 , 630 ± 1.734 and 116 ± 0.809 with total decrease of 24, 2, 156 and 34) mg/Kg dry weight basis was observed (Table 3.8 and Figure 3.8).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (B-5) at 1st stage of supplementation. Chromium content was also increased where magnesium and zinc were applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content was decreased where magnesium and chromium were applied alone and also in sub-box (A-1).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was decreased in sub-boxes (A-4) and (B-7), while increased in other sub-boxes. Marked increase was occurred in sub-box (C-10) where zinc was supplemented at 2nd stage.

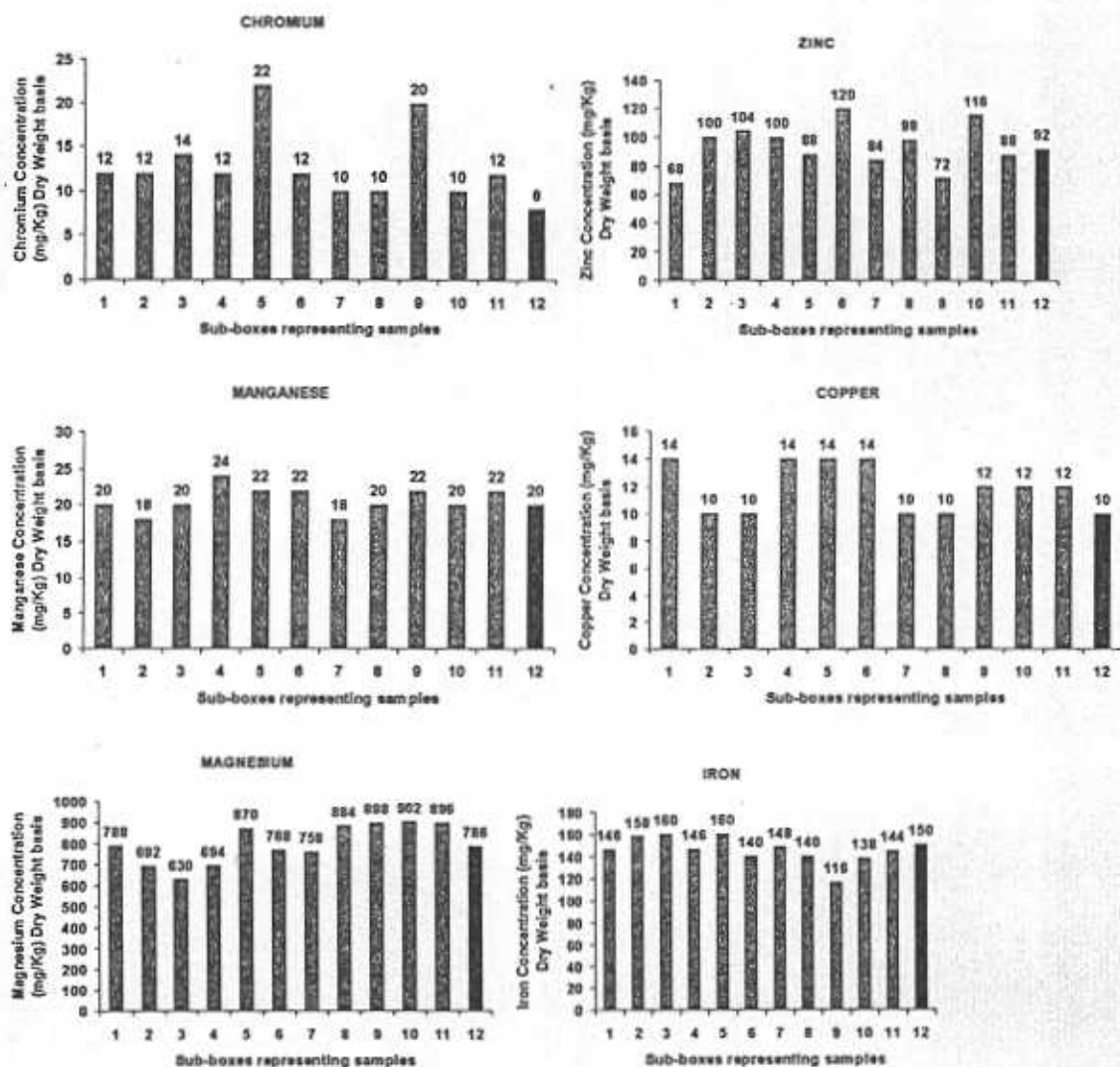
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Copper content was increased in both sub-boxes. Manganese and iron contents were decreased in sub-boxes (A-2), (A-4) and increased in sub-boxes (A-4), (A-3) respectively.

Table 3.8 *Allium-sativum*. (garlic)Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	12±0.057 C.V(%)=0.83 P-Value=0.0001	68±0.578 C.V(%)=1.47 P-Value=0.0001	20±0.173 C.V(%)=1.50 P-Value=0.0001	14±0.080 C.V(%)=1.00 P-Value=0.0001	788±1.734 C.V(%)=0.38 P-Value=0.0001	146±0.936 C.V(%)=1.10 P-Value=0.0001	↑	↓	N	↑	↑	↑
A-2	12±0.075 C.V(%)=1.08 P-Value=0.0001	100±0.867 C.V(%)=1.50 P-Value=0.0001	18±0.080 C.V(%)=0.77 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	692±2.312 C.V(%)=0.57 P-Value=0.0001	158±0.809 C.V(%)=0.88 P-Value=0.0001	↑	↑	↑	N	↑	↑
A-3	14±0.104 C.V(%)=1.28 P-Value=0.0001	104±0.693 C.V(%)=1.15 P-Value=0.0001	20±0.104 C.V(%)=0.90 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	630±1.734 C.V(%)=0.47 P-Value=0.0001	160±0.722 C.V(%)=0.78 P-Value=0.0001	↑	↑	N	N	↑	↑
A-4	12±0.080 C.V(%)=1.16 P-Value=0.0001	100±0.693 C.V(%)=1.20 P-Value=0.0001	24±0.173 C.V(%)=1.25 P-Value=0.0001	14±0.069 C.V(%)=0.85 P-Value=0.0001	694±1.734 C.V(%)=0.43 P-Value=0.0001	146±0.867 C.V(%)=1.027 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	22±0.173 C.V(%)=1.36 P-Value=0.0001	88±0.578 C.V(%)=1.13 P-Value=0.0001	22±0.115 C.V(%)=0.90 P-Value=0.0001	14±0.069 C.V(%)=0.85 P-Value=0.0001	870±2.890 C.V(%)=0.57 P-Value=0.0001	160±1.156 C.V(%)=1.25 P-Value=0.0001	↑	↓	↑	↑	↑	↑
B-6	12±0.069 C.V(%)=1.00 P-Value=0.0001	120±1.040 C.V(%)=1.50 P-Value=0.0001	22±0.115 C.V(%)=0.90 P-Value=0.0001	14±0.080 C.V(%)=1.00 P-Value=0.0001	768±2.312 C.V(%)=0.52 P-Value=0.0001	140±0.924 C.V(%)=1.14 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-7	10±0.046 C.V(%)=0.80 P-Value=0.0001	84±0.462 C.V(%)=0.95 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	758±1.156 C.V(%)=0.26 P-Value=0.0001	148±0.635 C.V(%)=0.74 P-Value=0.0001	↑	↓	↓	N	↑	↑
B-8	10±0.046 C.V(%)=0.80 P-Value=0.0001	98±0.462 C.V(%)=0.81 P-Value=0.0001	20±0.098 C.V(%)=0.85 P-Value=0.0001	10±0.040 C.V(%)=0.70 P-Value=0.0001	884±2.312 C.V(%)=0.45 P-Value=0.0001	140±1.040 C.V(%)=1.28 P-Value=0.0001	↑	↑	N	N	↑	↑
C-9	20±0.115 C.V(%)=1.00 P-Value=0.0001	72±0.404 C.V(%)=0.97 P-Value=0.0001	22±0.104 C.V(%)=0.81 P-Value=0.0001	12±0.046 C.V(%)=0.66 P-Value=0.0001	898±2.890 C.V(%)=0.55 P-Value=0.0001	116±0.809 C.V(%)=1.207 P-Value=0.0001	↑	↓	↑	↑	↑	↑
C-10	10±0.040 C.V(%)=0.70 P-Value=0.0001	116±0.924 C.V(%)=1.37 P-Value=0.0001	20±0.104 C.V(%)=0.90 P-Value=0.0001	12±0.057 C.V(%)=0.83 P-Value=0.0001	902±1.734 C.V(%)=0.33 P-Value=0.0001	138±0.838 C.V(%)=1.05 P-Value=0.0001	↑	↑	N	↑	↑	↑
C-11	12±0.063 C.V(%)=0.91 P-Value=0.0001	88±0.635 C.V(%)=1.25 P-Value=0.0001	22±0.104 C.V(%)=0.81 P-Value=0.0001	12±0.057 C.V(%)=0.83 P-Value=0.0001	896±2.890 C.V(%)=0.55 P-Value=0.0001	144±0.612 C.V(%)=0.73 P-Value=0.0001	↑	↓	↑	↑	↑	↑
C-12	8±0.028 C.V(%)=0.62 P-Value=0.0001	92±0.520 C.V(%)=0.97 P-Value=0.0001	20±0.098 C.V(%)=0.85 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	786±1.156 C.V(%)=0.25 P-Value=0.0001	150±0.693 C.V(%)=0.80 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.8
Sample. 7. *Allium-sativum*. (Garlic)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



SUMMER SEASON VEGETABLES

8. *Momordica-charantia*. (Bitter gourd)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (14 ± 0.086 , 104 ± 0.520 , 32 ± 0.104 , 10 ± 0.046 , 3836 ± 6.936 and 888 ± 1.156 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe (104 ± 0.924 , 156 ± 1.040 , 64 ± 0.462 , 50 ± 0.404 , 4012 ± 10.40 and 1396 ± 6.936) mg/Kg on dry weight basis in sub-boxes (B-5), (B-6), (A-4), (A-4), (A-2) and (A-4) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (90, 52, 32, 40, 176 and 508) mg/Kg dry weight basis. While in sub-boxes (A-1), (A-3, C-9), (C-10) and (C-10) minimum concentration of Zn, Mn, Mg and Fe (100 ± 0.664 , 24 ± 0.115 - 0.121 , 3358 ± 10.40 and 488 ± 3.468 with total decrease of 4, 8, 478 and 400) mg/Kg dry weight basis was observed (Table 3.9 and Figure 3.9).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (B-5) at 1st stage of supplementation. Chromium content was also increased where magnesium and zinc were applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content remained unchanged where magnesium and chromium were applied alone, except sub-box (C-9) in which Zn concentration decreased.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was decreased in all sub-boxes. Increase was observed in sub-boxes (A-2) and (A-3), where no magnesium was supplemented.

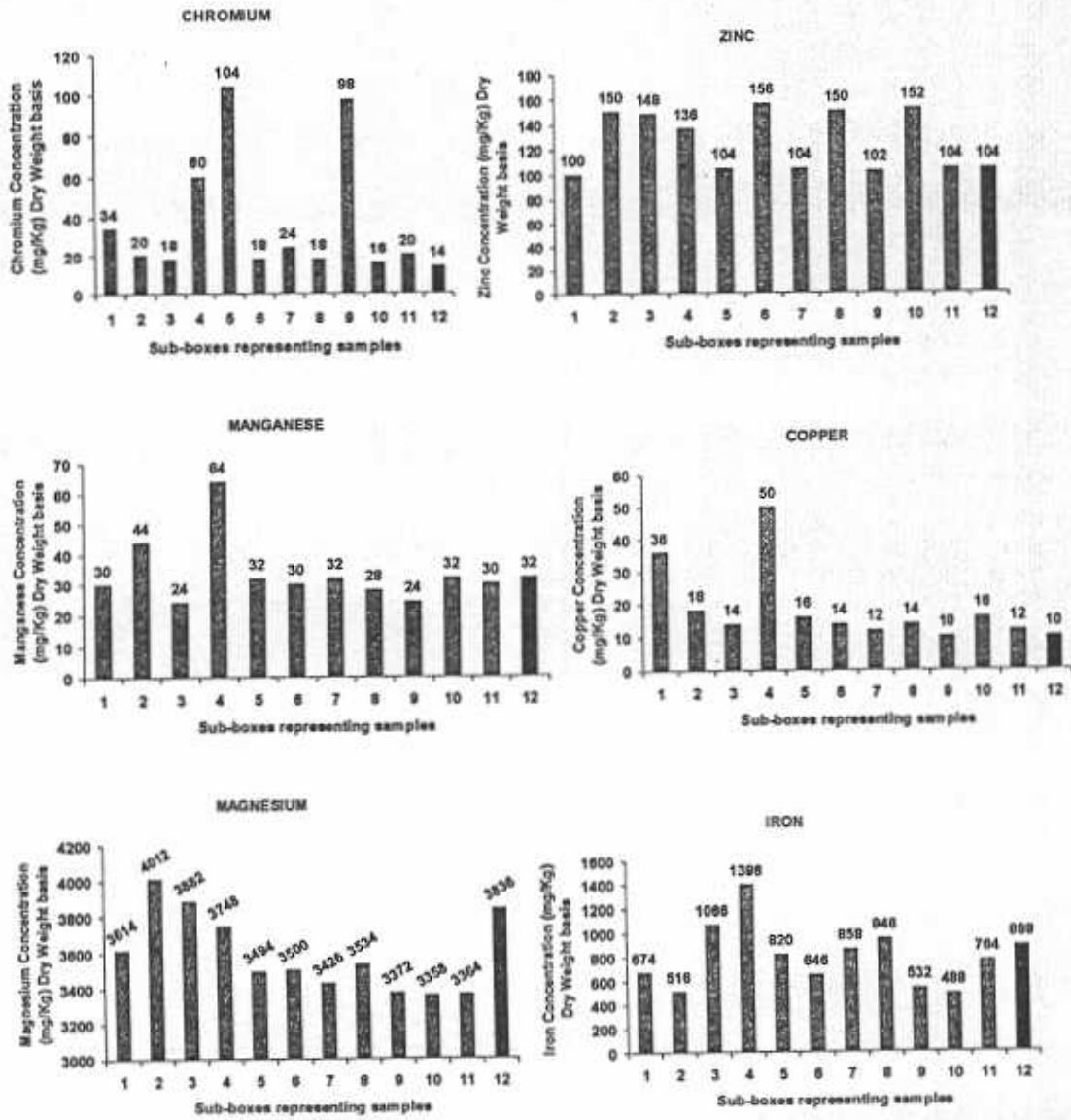
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper, manganese and iron was occurred in all sub-boxes with marked increase of all the three elements in sub-box (A-4).

Table 3.9 *Momordica-charantia*. (Bitter gourd) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-samples	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	34±0.173 C.V(%)=0.88 P-Value=0.0001	100±0.664 C.V(%)=1.15 P-Value=0.0001	30±0.144 C.V(%)=0.83 P-Value=0.0001	36±0.115 C.V(%)=0.55 P-Value=0.0001	3614±5.780 C.V(%)=0.27 P-Value=0.0001	674±1.734 C.V(%)=0.44 P-Value=0.0001	↑	↓	↑	↑	↓	↓
A-2	20±0.121 C.V(%)=1.05 P-Value=0.0001	150±1.156 C.V(%)=1.33 P-Value=0.0001	44±0.173 C.V(%)=0.68 P-Value=0.0001	18±0.104 C.V(%)=1.00 P-Value=0.0001	4012±10.40 C.V(%)=0.44 P-Value=0.0001	516±2.312 C.V(%)=0.77 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-3	18±0.080 C.V(%)=0.77 P-Value=0.0001	148±0.693 C.V(%)=0.81 P-Value=0.0001	24±0.121 C.V(%)=0.87 P-Value=0.0001	14±0.075 C.V(%)=0.92 P-Value=0.0001	3882±15.02 C.V(%)=0.66 P-Value=0.0001	1066±5.780 C.V(%)=0.93 P-Value=0.0001	↑	↑	↓	↑	↑	↑
A-4	60±0.462 C.V(%)=1.33 P-Value=0.0001	136±0.635 C.V(%)=0.80 P-Value=0.0001	64±0.462 C.V(%)=1.25 P-Value=0.0001	50±0.404 C.V(%)=1.40 P-Value=0.0001	3748±12.71 C.V(%)=0.58 P-Value=0.0001	1396±6.936 C.V(%)=0.85 P-Value=0.0001	↑	↑	↑	↑	↓	↑
B-5	104±0.924 C.V(%)=1.53 P-Value=0.0001	104±0.462 C.V(%)=0.76 P-Value=0.0001	32±0.115 C.V(%)=0.62 P-Value=0.0001	16±0.063 C.V(%)=0.68 P-Value=0.0001	3494±6.936 C.V(%)=0.34 P-Value=0.0001	820±3.468 C.V(%)=0.73 P-Value=0.0001	↑	N	N	↑	↓	↓
B-6	18±0.075 C.V(%)=0.72 P-Value=0.0001	156±1.040 C.V(%)=1.15 P-Value=0.0001	30±0.115 C.V(%)=0.66 P-Value=0.0001	14±0.057 C.V(%)=0.71 P-Value=0.0001	3500±6.936 C.V(%)=0.34 P-Value=0.0001	646±2.312 C.V(%)=0.61 P-Value=0.0001	↑	↑	↓	↑	↓	↓
B-7	24±0.144 C.V(%)=1.04 P-Value=0.0001	104±0.404 C.V(%)=0.67 P-Value=0.0001	32±0.104 C.V(%)=0.56 P-Value=0.0001	12±0.080 C.V(%)=1.16 P-Value=0.0001	3426±9.248 C.V(%)=0.46 P-Value=0.0001	858±6.936 C.V(%)=1.39 P-Value=0.0001	↑	N	N	↑	↓	↑
B-8	18±0.085 C.V(%)=0.83 P-Value=0.0001	150±0.693 C.V(%)=0.80 P-Value=0.0001	28±0.086 C.V(%)=0.53 P-Value=0.0001	14±0.063 C.V(%)=0.78 P-Value=0.0001	3534±5.780 C.V(%)=0.28 P-Value=0.0001	946±10.40 C.V(%)=1.90 P-Value=0.0001	↑	↑	↓	↑	↓	↑
C-9	98±0.520 C.V(%)=0.91 P-Value=0.0001	102±0.404 C.V(%)=0.68 P-Value=0.0001	24±0.115 C.V(%)=0.83 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	3372±20.80 C.V(%)=1.06 P-Value=0.0001	532±1.734 C.V(%)=0.56 P-Value=0.0001	↑	↑	↓	N	↓	↓
C-10	16±0.075 C.V(%)=0.81 P-Value=0.0001	152±0.924 C.V(%)=1.05 P-Value=0.0001	32±0.104 C.V(%)=0.56 P-Value=0.0001	16±0.069 C.V(%)=0.75 P-Value=0.0001	3358±10.40 C.V(%)=0.53 P-Value=0.0001	488±3.468 C.V(%)=1.22 P-Value=0.0001	↑	↑	N	↑	↓	↓
C-11	20±0.115 C.V(%)=1.00 P-Value=0.0001	104±0.520 C.V(%)=0.86 P-Value=0.0001	30±0.104 C.V(%)=0.60 P-Value=0.0001	12±0.069 C.V(%)=1.00 P-Value=0.0001	3364±10.40 C.V(%)=0.53 P-Value=0.0001	764±2.312 C.V(%)=0.52 P-Value=0.0001	↑	N	↓	↑	↓	↓
C-12	14±0.086 C.V(%)=1.07 P-Value=0.0001	104±0.520 C.V(%)=0.86 P-Value=0.0001	32±0.104 C.V(%)=0.56 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	3836±6.936 C.V(%)=0.31 P-Value=0.0001	888±1.156 C.V(%)=0.22 P-Value=0.0001	C	C	C	C	C	C

N= No Increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.9
Sample. 8. *Momordica-charantia* (Bitter gourd)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



9. *Luffa acutangula*. (Bath sponge)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (ND, 84 ± 0.404 , 28 ± 0.173 , 16 ± 0.080 , 2900 ± 6.936 and 364 ± 1.734 with a maximum concentration of Cr, Zn, Mn, Cu and Mg (14 ± 0.069 , 188 ± 1.156 , 38 ± 0.202 - 0.231 , 36 ± 0.144 and 3862 ± 16.18) mg/Kg on dry weight basis in sub-boxes (B-5), (B-6), (A-2, A-4), (A-4) and (A-1) respectively. The total increase of Cr, Zn, Mn, Cu and Mg recorded was (14, 104, 10, 20 and 962) mg/Kg dry weight basis. While in sub-boxes (B-7), (B-7), (A-3, B-5), (C-9) and (C-11) minimum concentration of Zn, Mn, Cu, Mg and Fe (76 ± 0.578 , 24 ± 0.231 , 14 ± 0.057 - 0.086 , 2790 ± 8.092 and 204 ± 1.156 with total decrease of 8, 4, 2, 110 and 160) mg/Kg dry weight basis was observed (Table 3.10 & Figure 3.10).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (Com-1, 2, 3, 4, 8) and individually (Ind-15, 9). Increase in chromium content was occurred in all sub-boxes with significant increase in sub-box (B-5) at 1st stage of supplementation. Chromium content did not show any increase or decrease, where magnesium and zinc were applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content decreased where magnesium and chromium were applied alone.

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes except sub-box (B-8) with significant increase in sub-box (A-1) at 1st stage of supplementation. The concentration was increased in sub-boxes supplemented with zinc; while in case of chromium there was observed increase in one sub-box (B-5) and decrease in other sub-box (C-9).

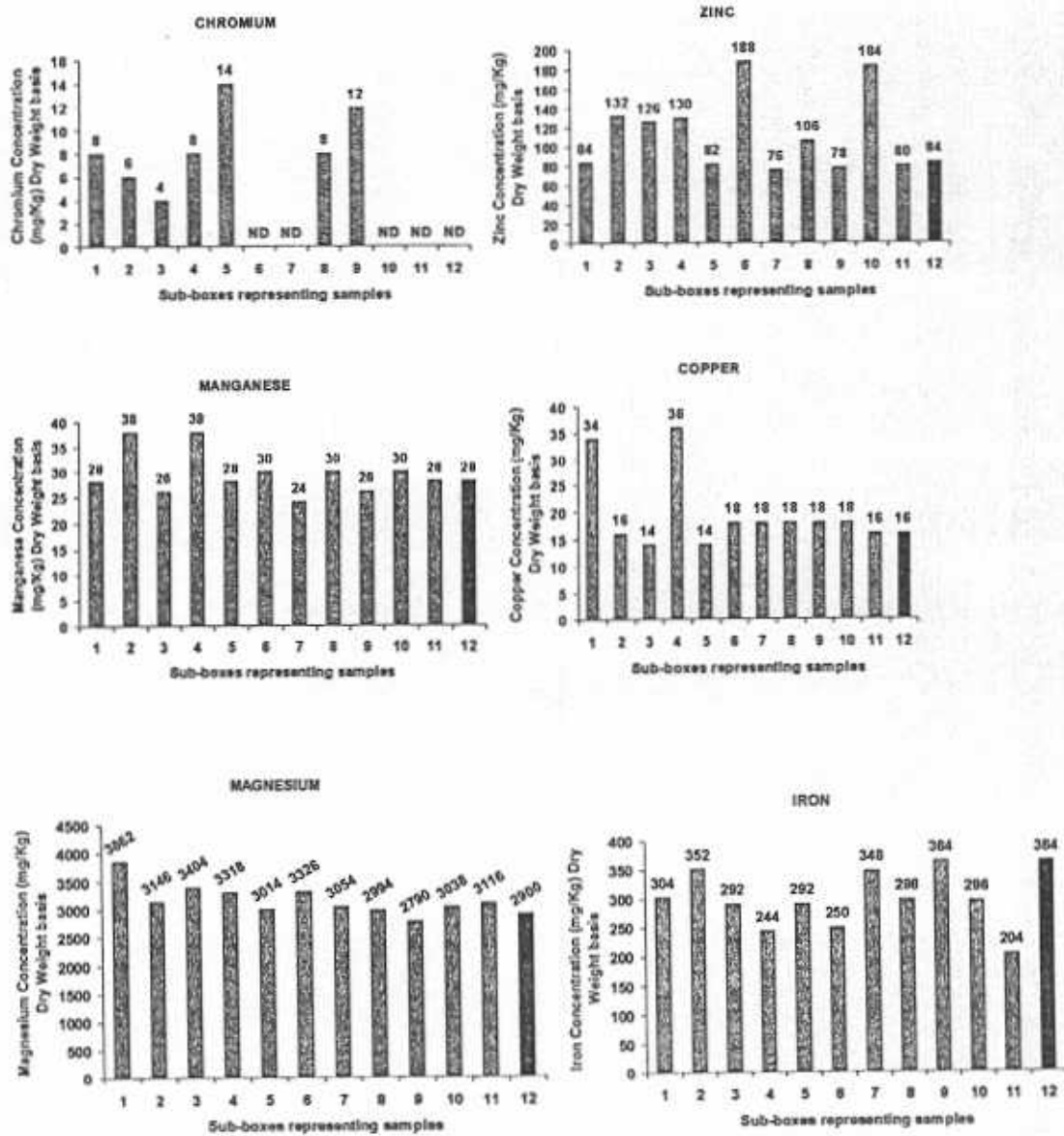
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. The iron content was decreased, while copper and manganese contents were increased in both sub-boxes with marked increase in sub-boxes (A-4) and (A-2, A-4) respectively.

Table 3.10 *Luffa acutangula*. (Bath sponge) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-samples	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
							Decrease(↓)/ Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	8±0.028 C.V(%)=0.62 P-Value=0.0001	84±0.462 C.V(%)=0.95 P-Value=0.0001	28±0.121 C.V(%)=0.75 P-Value=0.0001	34±0.121 C.V(%)=0.61 P-Value=0.0001	3862±16.18 C.V(%)=0.72 P-Value=0.0001	304±1.734 C.V(%)=0.98 P-Value=0.0001	↑	N	N	↑	↑	↑
A-2	6±0.023 C.V(%)=0.66 P-Value=0.0001	132±0.635 C.V(%)=0.83 P-Value=0.0001	38±0.202 C.V(%)=0.92 P-Value=0.0001	16±0.080 C.V(%)=0.87 P-Value=0.0001	3146±10.40 C.V(%)=0.57 P-Value=0.0001	352±2.312 C.V(%)=1.13 P-Value=0.0001	↑	↑	↑	N	↑	↑
A-3	4±0.011 C.V(%)=0.50 P-Value=0.0001	126±0.520 C.V(%)=0.71 P-Value=0.0001	26±0.144 C.V(%)=0.96 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	3404±8.092 C.V(%)=0.41 P-Value=0.0001	292±2.312 C.V(%)=1.36 P-Value=0.0001	↑	↑	↓	↑	↑	↑
A-4	8±0.028 C.V(%)=0.62 P-Value=0.0001	130±0.520 C.V(%)=0.69 P-Value=0.0001	38±0.231 C.V(%)=1.05 P-Value=0.0001	36±0.144 C.V(%)=0.69 P-Value=0.0001	3318±16.18 C.V(%)=0.84 P-Value=0.0001	244±2.890 C.V(%)=2.04 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	14±0.069 C.V(%)=0.85 P-Value=0.0001	82±0.462 C.V(%)=0.97 P-Value=0.0001	28±0.098 C.V(%)=0.60 P-Value=0.0001	14±0.057 C.V(%)=0.71 P-Value=0.0001	3014±8.092 C.V(%)=0.46 P-Value=0.0001	292±1.734 C.V(%)=1.02 P-Value=0.0001	↑	↓	N	↑	↑	↑
B-6	ND	188±1.156 C.V(%)=1.06 P-Value=0.0001	30±0.231 C.V(%)=1.33 P-Value=0.0001	18±0.080 C.V(%)=0.77 P-Value=0.0001	3326±26.58 C.V(%)=1.38 P-Value=0.0001	250±2.312 C.V(%)=1.60 P-Value=0.0001	N	↑	↑	↑	↑	↑
B-7	ND	76±0.578 C.V(%)=1.31 P-Value=0.0001	24±0.231 C.V(%)=1.66 P-Value=0.0001	18±0.080 C.V(%)=0.77 P-Value=0.0001	3054±10.40 C.V(%)=0.58 P-Value=0.0001	348±1.734 C.V(%)=0.86 P-Value=0.0001	N	↓	↓	↑	↑	↑
B-8	8±0.023 C.V(%)=0.50 P-Value=0.0001	106±0.520 C.V(%)=0.84 P-Value=0.0001	30±0.289 C.V(%)=1.66 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	2994±30.05 C.V(%)=1.73 P-Value=0.0001	298±1.734 C.V(%)=1.00 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-9	12±0.057 C.V(%)=0.83 P-Value=0.0001	78±0.404 C.V(%)=0.89 P-Value=0.0001	26±0.173 C.V(%)=1.15 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	2790±8.092 C.V(%)=0.50 P-Value=0.0001	364±2.890 C.V(%)=1.37 P-Value=0.0001	↑	↓	↓	↑	↑	N
C-10	ND	184±0.924 C.V(%)=0.86 P-Value=0.0001	30±0.231 C.V(%)=1.33 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	3038±9.248 C.V(%)=0.52 P-Value=0.0001	296±2.312 C.V(%)=1.35 P-Value=0.0001	N	↑	↑	↑	↑	↑
C-11	ND	80±0.404 C.V(%)=0.87 P-Value=0.0001	28±0.173 C.V(%)=1.07 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	3116±15.02 C.V(%)=0.83 P-Value=0.0001	204±1.156 C.V(%)=0.98 P-Value=0.0001	N	↓	N	N	↑	↑
C-12	ND	84±0.404 C.V(%)=0.83 P-Value=0.0001	28±0.173 C.V(%)=1.07 P-Value=0.0001	16±0.080 C.V(%)=0.87 P-Value=0.0001	2900±6.936 C.V(%)=0.41 P-Value=0.0001	364±1.734 C.V(%)=0.82 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.10
 Sample .9. *Luffa-acutangula* (Bath sponge)
 Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



10. *Solanum-melongena*. (Bringal/egg-plant)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (8 ± 0.052 , 92 ± 0.693 , 30 ± 0.289 , 24 ± 0.231 , 2152 ± 9.248 and 236 ± 2.312 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe (12 ± 0.115 - 0.144 , 200 ± 1.734 , 40 ± 0.231 , 36 ± 0.202 , 2826 ± 6.936 and 292 ± 1.156) mg/Kg on dry weight basis in sub-boxes (B-5,B-7), (B-6), (A-4), (A-4),(B-5) and (A-4) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (4, 108, 10, 12, 134 and 56) mg/Kg dry weight basis. While in sub-boxes (A-3), (C-9), (B-8,C-11), (C-11) and (B-5) minimum concentration of Cr, Zn, Mn, Cu and Fe (6 ± 0.028 , 72 ± 0.404 , 26 ± 0.115 - 0.144 , 16 ± 0.057 and 166 ± 2.890 with total decrease of 2, 20, 4, 8 and 70) mg/Kg dry weight basis was observed (Table 3.11 and Figure 3.11).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). No increase or decrease of chromium content in sub-boxes (A-1, A-2), decrease in sub-box (A-3) and increase in sub-boxes (A-4,B-5,B-8,C-9) was observed. Marked increase in sub-box (B-5) at 1st stage of supplementation was recorded. Chromium content was increased and remained unchanged where magnesium and zinc were applied respectively.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content decreased where magnesium and chromium were applied alone and remained unchanged in sub-box (A-1).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes with marked increase in sub-box (B-5) at 1st stage of supplementation. The concentration was increased in sub-boxes supplemented with chromium. Increase was occurred in zinc supplemented sub-boxes also.

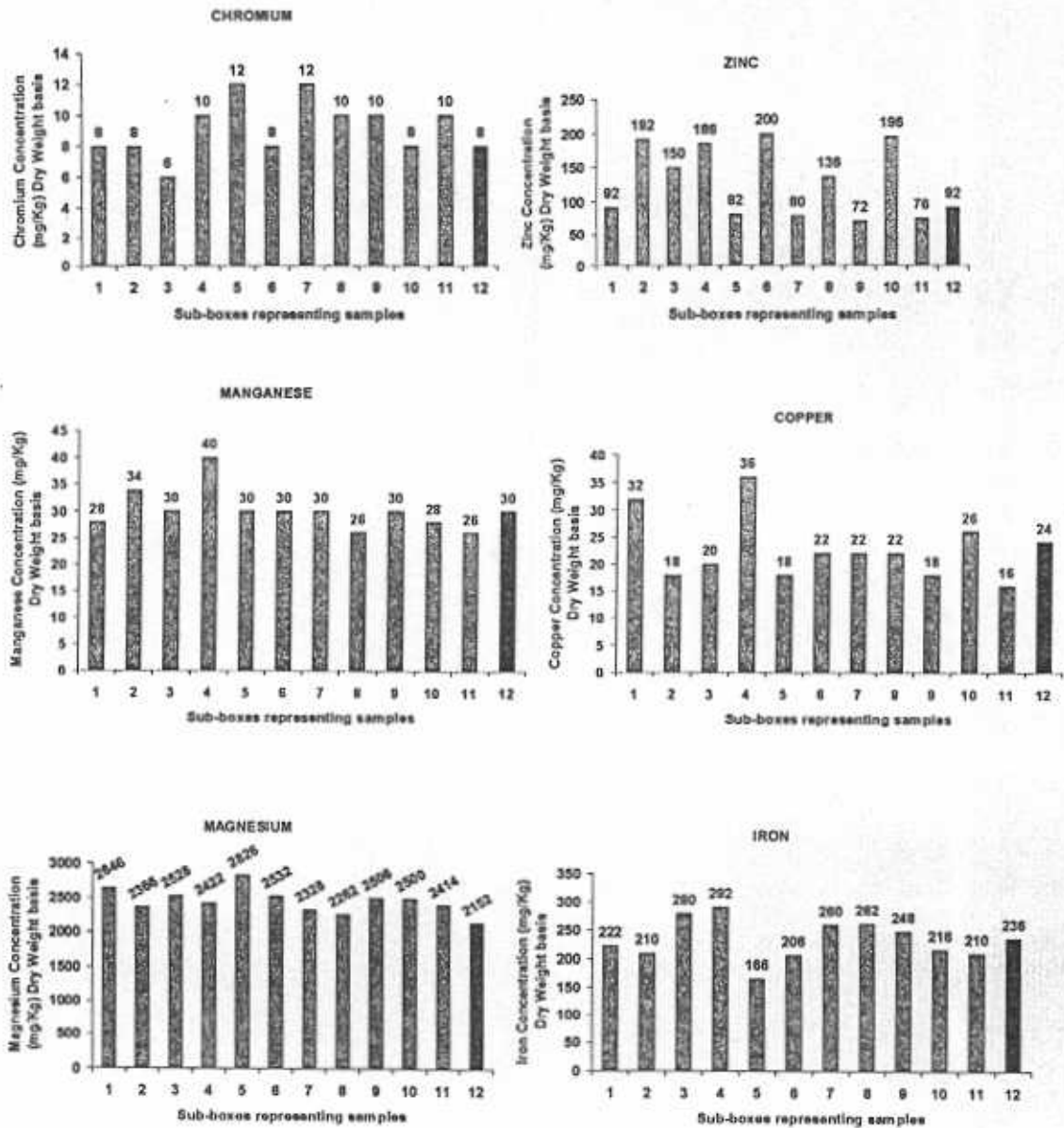
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper, manganese and iron was occurred in all sub-boxes with marked increase of all the three in sub-box (A-4).

Table 3.11 *Solanum melongena*. (Brinjal, egg-plant) Concentration of Cr, Zn, Mn, Cu, Mg and Fe (mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
							Decrease(↓) / Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	8±0.052 C.V(%)=1.12 P-Value=0.0001	92±0.462 C.V(%)=0.86 P-Value=0.0001	28±0.144 C.V(%)=0.89 P-Value=0.0001	32±0.121 C.V(%)=0.65 P-Value=0.0001	26±6±12.71 C.V(%)=0.83 P-Value=0.0001	222±2.312 C.V(%)=1.80 P-Value=0.0001	N	N	↓	↑	↑	↑
A-2	8±0.057 C.V(%)=1.25 P-Value=0.0001	192±1.156 C.V(%)=1.04 P-Value=0.0001	34±0.173 C.V(%)=0.88 P-Value=0.0001	18±0.080 C.V(%)=0.77 P-Value=0.0001	236±6±10.40 C.V(%)=0.76 P-Value=0.0001	210±3.468 C.V(%)=2.85 P-Value=0.0003	N	↑	↑	↓	↑	↓
A-3	6±0.028 C.V(%)=0.83 P-Value=0.0001	150±1.040 C.V(%)=1.20 P-Value=0.0001	30±0.098 C.V(%)=0.56 P-Value=0.0001	20±0.098 C.V(%)=0.85 P-Value=0.0001	252±8±15.02 C.V(%)=1.02 P-Value=0.0001	280±1.734 C.V(%)=1.07 P-Value=0.0001	↓	↑	N	↓	↑	↑
A-4	10±0.057 C.V(%)=1.00 P-Value=0.0001	186±1.040 C.V(%)=0.96 P-Value=0.0001	40±0.231 C.V(%)=1.00 P-Value=0.0001	36±0.202 C.V(%)=0.97 P-Value=0.0001	242±2±15.02 C.V(%)=1.07 P-Value=0.0001	292±1.156 C.V(%)=0.68 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	12±0.115 C.V(%)=1.66 P-Value=0.0001	82±0.578 C.V(%)=1.21 P-Value=0.0001	30±0.289 C.V(%)=1.66 P-Value=0.0001	18±0.080 C.V(%)=0.77 P-Value=0.0001	282±6±9.36 C.V(%)=0.42 P-Value=0.0001	166±2.890 C.V(%)=3.01 P-Value=0.0003	↑	↓	N	↓	↑	↓
B-6	8±0.052 C.V(%)=1.12 P-Value=0.0001	200±1.734 C.V(%)=1.50 P-Value=0.0001	30±0.231 C.V(%)=1.35 P-Value=0.0001	22±0.173 C.V(%)=1.36 P-Value=0.0001	232±6±9.36 C.V(%)=0.47 P-Value=0.0001	206±2.890 C.V(%)=2.42 P-Value=0.0002	N	↑	N	↓	↑	↓
B-7	12±0.144 C.V(%)=2.08 P-Value=0.0001	80±0.462 C.V(%)=1.00 P-Value=0.0001	30±0.231 C.V(%)=1.33 P-Value=0.0001	22±0.173 C.V(%)=1.36 P-Value=0.0001	232±8±21.96 C.V(%)=1.63 P-Value=0.0001	260±1.734 C.V(%)=1.15 P-Value=0.0001	↑	↓	N	↓	↑	↑
B-8	10±0.057 C.V(%)=1.00 P-Value=0.0001	136±0.924 C.V(%)=1.17 P-Value=0.0001	26±0.144 C.V(%)=0.96 P-Value=0.0001	22±0.173 C.V(%)=1.36 P-Value=0.0001	226±2±13.87 C.V(%)=1.06 P-Value=0.0001	262±1.734 C.V(%)=1.14 P-Value=0.0001	↑	↑	↓	↓	↑	↑
C-9	10±0.052 C.V(%)=0.90 P-Value=0.0001	72±0.404 C.V(%)=0.97 P-Value=0.0001	30±0.231 C.V(%)=1.33 P-Value=0.0001	18±0.086 C.V(%)=0.83 P-Value=0.0001	250±6±12.71 C.V(%)=0.87 P-Value=0.0001	248±2.890 C.V(%)=2.01 P-Value=0.0001	↑	↓	N	↓	↑	↑
C-10	8±0.052 C.V(%)=1.12 P-Value=0.0001	196±1.040 C.V(%)=0.91 P-Value=0.0001	28±0.121 C.V(%)=0.75 P-Value=0.0001	26±0.173 C.V(%)=1.15 P-Value=0.0001	250±0±11.56 C.V(%)=0.80 P-Value=0.0001	216±1.156 C.V(%)=0.92 P-Value=0.0001	N	↑	↓	↑	↑	↓
C-11	10±0.052 C.V(%)=0.90 P-Value=0.0001	76±0.404 C.V(%)=0.92 P-Value=0.0001	26±0.115 C.V(%)=0.76 P-Value=0.0001	16±0.057 C.V(%)=0.62 P-Value=0.0001	241±4±33.52 C.V(%)=2.40 P-Value=0.0002	210±1.156 C.V(%)=0.95 P-Value=0.0001	↑	↓	↓	↓	↑	↓
C-12	8±0.052 C.V(%)=1.12 P-Value=0.0001	92±0.693 C.V(%)=1.30 P-Value=0.0001	30±0.289 C.V(%)=1.66 P-Value=0.0001	24±0.231 C.V(%)=1.66 P-Value=0.0001	215±2±9.248 C.V(%)=0.74 P-Value=0.0001	236±2.312 C.V(%)=1.69 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.11
Sample .10. *Solanum-melongena*. (Brinjal)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



11. *Portulaca-oleracea*. (Pot purslane)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (12 ± 0.080 , 290 ± 2.312 , 158 ± 1.040 , 16 ± 0.086 , 24180 ± 16.18 and 2768 ± 23.12 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe (24 ± 0.173 , 352 ± 1.156 , 184 ± 1.040 , 36 ± 0.173 , 24840 ± 13.87 and 2792 ± 12.71) mg/Kg on dry weight basis in sub-boxes (B-5), (B-6), (A-2), (A-1), (A-1) and (A-4) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (12, 62, 26, 20, 660 and 24) mg/Kg dry weight basis. While in sub-boxes (B-6), (A-1), (B-6), (A-2), (B-7) and (B-8) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (10 ± 0.046 , 142 ± 0.693 , 112 ± 0.664 , 10 ± 0.046 , 15560 ± 20.80 and 2208 ± 26.58 with total decrease of 2, 148, 46, 6, 8620 and 560) mg/Kg dry weight basis was observed (Table 3.12 and Figure 3.12).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with no increase or decrease in sub-box (A-3) and marked increase in sub-box (B-5) at 1st stage of supplementation. Chromium content was decreased in sub-box (B-6) and increased in sub-box (C-10) in case of zinc supplementation. Similarly, in case of magnesium increased occurred in sub-box (B-7) and no change in sub-box (C-11).

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content decreased where magnesium and chromium were applied alone and also in sub-box (A-1).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in sub-boxes (A-1, B-8) and decreased in all other sub-boxes. Marked increase occurred in sub-boxes (B-8) at 1st stage of supplementation. The concentration was decreased in sub-boxes supplemented with chromium and zinc.

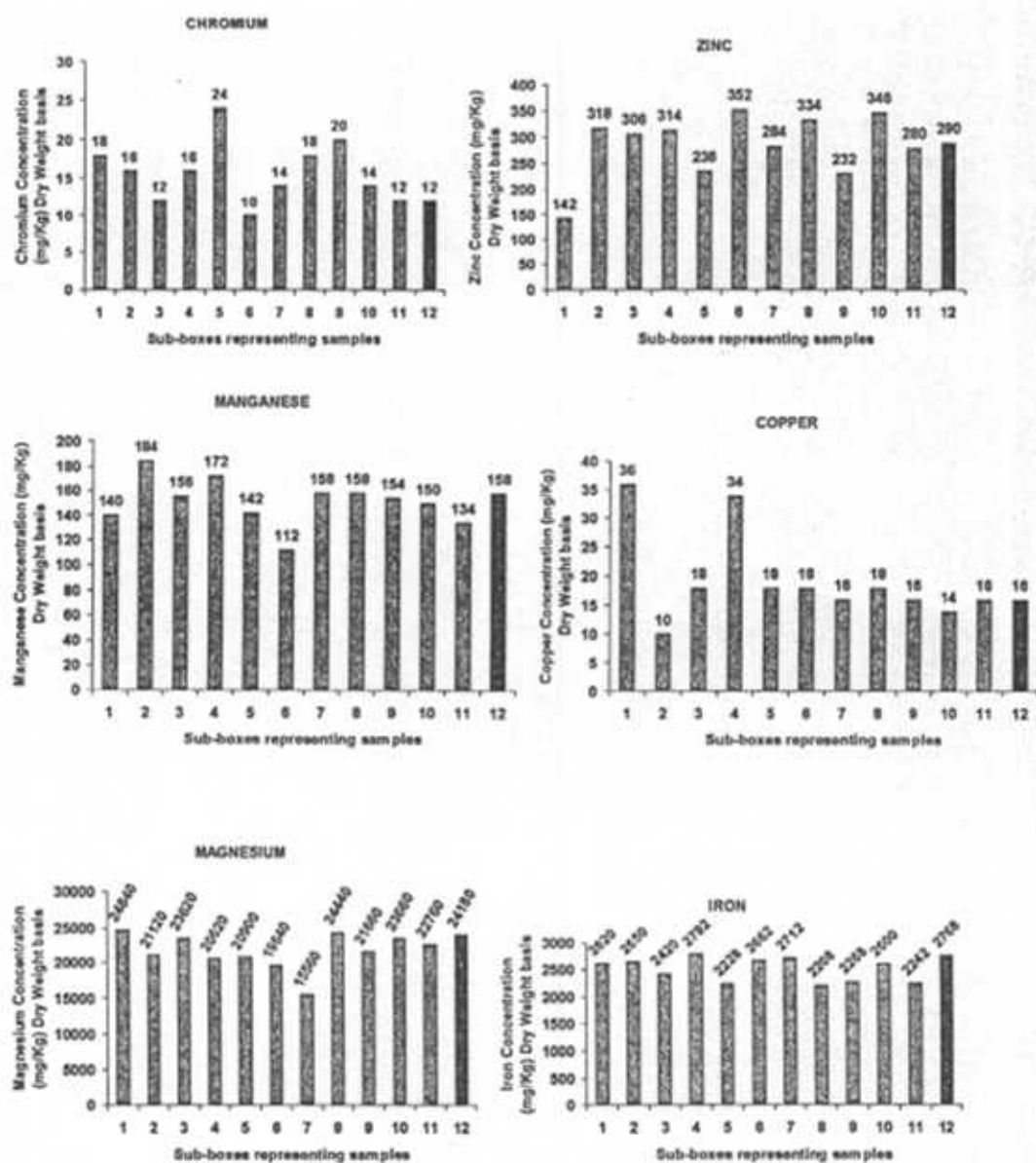
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper and manganese was occurred in all sub-boxes with marked increase in sub-box (A-1) and (A-2) respectively. Iron content was decreased in sub-box (3) and increased in sub-box (A-4).

Table 3.12 *Portulaca-oleracea*. (Pot purslane) Concentration of Cr, Zn, Mn, Cu, Mg and Fe (mg/kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination Decrease(↓)/ Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	18±0.115 C.V(%)=1.11 P-Value=0.0001	142±0.693 C.V(%)=0.84 P-Value=0.0001	140±0.693 C.V(%)=0.85 P-Value=0.0001	36±0.173 C.V(%)=0.83 P-Value=0.0001	24840±13.87 C.V(%)=0.09 P-Value=0.0001	2620±15.02 C.V(%)=0.99 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-2	16±0.104 C.V(%)=1.12 P-Value=0.0001	318±1.156 C.V(%)=0.62 P-Value=0.0001	184±1.040 C.V(%)=0.97 P-Value=0.0001	10±0.046 C.V(%)=0.80 P-Value=0.0001	21120±5.780 C.V(%)=0.04 P-Value=0.0001	2650±18.49 C.V(%)=1.20 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-3	12±0.080 C.V(%)=1.16 P-Value=0.0001	306±2.312 C.V(%)=1.30 P-Value=0.0001	156±0.867 C.V(%)=0.96 P-Value=0.0001	18±0.104 C.V(%)=1.00 P-Value=0.0001	23620±15.02 C.V(%)=0.11 P-Value=0.0001	2420±10.40 C.V(%)=0.74 P-Value=0.0001	N	↑	↑	↑	↑	↑
A-4	16±0.098 C.V(%)=1.06 P-Value=0.0001	314±1.156 C.V(%)=0.63 P-Value=0.0001	172±0.924 C.V(%)=0.93 P-Value=0.0001	34±0.144 C.V(%)=0.73 P-Value=0.0001	20620±10.40 C.V(%)=0.08 P-Value=0.0001	2792±12.71 C.V(%)=0.78 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	24±0.173 C.V(%)=1.25 P-Value=0.0001	236±1.156 C.V(%)=0.84 P-Value=0.0001	142±0.693 C.V(%)=0.84 P-Value=0.0001	18±0.104 C.V(%)=1.00 P-Value=0.0001	20900±12.71 C.V(%)=0.10 P-Value=0.0001	2228±24.27 C.V(%)=1.88 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-6	10±0.046 C.V(%)=0.80 P-Value=0.0001	352±1.156 C.V(%)=0.56 P-Value=0.0001	112±0.664 C.V(%)=1.02 P-Value=0.0001	18±0.104 C.V(%)=1.00 P-Value=0.0001	19640±9.248 C.V(%)=0.08 P-Value=0.0001	2662±6.936 C.V(%)=0.45 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-7	14±0.086 C.V(%)=1.07 P-Value=0.0001	284±2.312 C.V(%)=1.40 P-Value=0.0001	158±0.982 C.V(%)=1.07 P-Value=0.0001	16±0.080 C.V(%)=0.87 P-Value=0.0001	15560±20.80 C.V(%)=0.23 P-Value=0.0001	2712±18.49 C.V(%)=1.17 P-Value=0.0001	↑	↑	N	N	↑	↑
B-8	18±0.109 C.V(%)=1.05 P-Value=0.0001	334±2.312 C.V(%)=1.19 P-Value=0.0001	158±1.040 C.V(%)=1.13 P-Value=0.0001	18±0.104 C.V(%)=1.00 P-Value=0.0001	24440±12.71 C.V(%)=0.09 P-Value=0.0001	2208±26.58 C.V(%)=2.08 P-Value=0.0001	↑	↑	N	↑	↑	↑
C-9	20±0.150 C.V(%)=1.30 P-Value=0.0001	232±1.156 C.V(%)=0.86 P-Value=0.0001	154±0.809 C.V(%)=0.90 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	21660±10.40 C.V(%)=0.08 P-Value=0.0001	2268±6.936 C.V(%)=0.52 P-Value=0.0001	↑	↑	↑	N	↑	↑
C-10	14±0.086 C.V(%)=1.07 P-Value=0.0001	346±1.156 C.V(%)=0.57 P-Value=0.0001	150±0.693 C.V(%)=0.80 P-Value=0.0001	14±0.069 C.V(%)=0.85 P-Value=0.0001	23660±10.40 C.V(%)=0.07 P-Value=0.0001	2600±20.80 C.V(%)=1.38 P-Value=0.0001	↑	↑	↑	↑	↑	↑
C-11	12±0.080 C.V(%)=1.16 P-Value=0.0001	280±2.312 C.V(%)=1.42 P-Value=0.0001	134±0.635 C.V(%)=0.82 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	22760±9.248 C.V(%)=0.07 P-Value=0.0001	2242±6.936 C.V(%)=0.53 P-Value=0.0001	N	↑	↑	N	↑	↑
C-12	12±0.080 C.V(%)=1.16 P-Value=0.0001	290±2.312 C.V(%)=1.37 P-Value=0.0001	158±1.040 C.V(%)=1.13 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	24180±16.18 C.V(%)=0.11 P-Value=0.0001	2768±23.12 C.V(%)=1.44 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.12
Sample.11. *Portulaca-oleracea* (Pot purslane/Kolfa)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



12. *Spinacea-oleracea*(Spinach)

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was (26 ± 0.115 , 128 ± 0.693 , 734 ± 3.468 , 16 ± 0.098 , 14120 ± 12.71 and 2852 ± 10.40 with a maximum concentration of Cr, Zn, Cu, Mg and Fe (116 ± 1.60 , 364 ± 3.00 , 24 ± 0.173 , 22920 ± 16.18 and 3994 ± 9.248) mg/Kg on dry weight basis in sub-boxes (B-5), (B-6), (A-4), (A-1) and (A-4) respectively. The total increase of Cr, Zn, Cu, Mg and Fe recorded was (90, 236, 8, 8800 and 1142) mg/Kg dry weight basis. While in sub-boxes (C-11), (A-1), (C-9), (A-3,B-7,B-8,C-10), (B-5) and (B-5) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe (18 ± 0.098 , 100 ± 0.664 , 216 ± 2.890 , $14\pm0.080-0.086$, 8160 ± 8.092 and 2626 ± 23.12 with total decrease of 8, 264, 518, 2, 5960 and 226) mg/Kg dry weight basis was observed (Table 3.13 and Figure 3.13).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (B-5) at 1st stage of supplementation. Chromium content was decreased where magnesium and zinc were applied alone.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (B-6) at 1st stage of supplementation. Zinc content decreased where magnesium and chromium were applied alone and in sub-box (A-1). In case of chromium supplementation no change occurred in sub-box (B-5)

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes. Marked increase occurred in sub-box (A-1) at 1st stage of supplementation. The concentration was decreased in chromium and zinc supplemented sub-boxes with only exception of increase in sub-box (C-9).

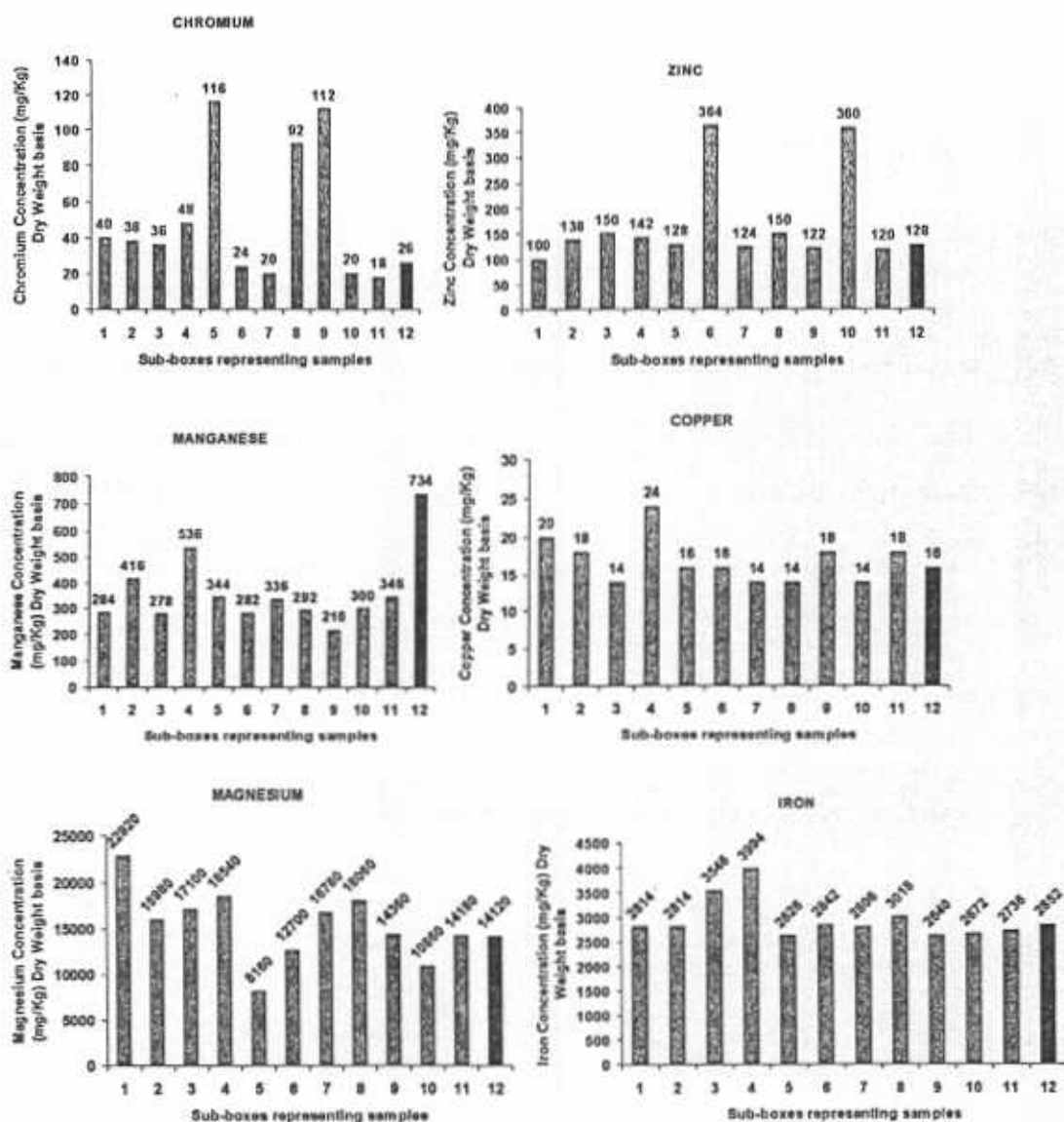
Copper, manganese and iron were applied in the mixtures (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper and iron was occurred in all samples with marked increase in samples (4) and sample (4) respectively. Manganese content was decreased in both samples.

Table 3.13 *Spinacea-oleracea* (Spinach) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	40±0.289 C.V(%)=1.25 P-Value=0.0001	100±0.664 C.V(%)=1.15 P-Value=0.0001	284±4.624 C.V(%)=2.81 P-Value=0.0001	20±0.115 C.V(%)=1.00 P-Value=0.0001	22920±16.18 C.V(%)=0.12 P-Value=0.0001	2814±9.248 C.V(%)=0.56 P-Value=0.0001	↑	↓	↓	↑	↑	↓
A-2	38±0.231 C.V(%)=1.05 P-Value=0.0001	138±0.635 C.V(%)=0.79 P-Value=0.0001	416±1.156 C.V(%)=0.48 P-Value=0.0001	18±0.109 C.V(%)=1.05 P-Value=0.0001	15980±6.936 C.V(%)=0.07 P-Value=0.0001	2814±31.21 C.V(%)=1.91 P-Value=0.0001	↑	↑	↓	↑	↑	↓
A-3	36±0.144 C.V(%)=0.69 P-Value=0.0001	150±1.040 C.V(%)=1.20 P-Value=0.0001	278±1.156 C.V(%)=0.71 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	17100±10.40 C.V(%)=0.10 P-Value=0.0001	3546±38.15 C.V(%)=1.86 P-Value=0.0001	↑	↑	↓	↓	↑	↑
A-4	48±0.173 C.V(%)=0.62 P-Value=0.0001	142±0.693 C.V(%)=0.84 P-Value=0.0001	536±3.468 C.V(%)=1.11 P-Value=0.0001	24±0.173 C.V(%)=1.25 P-Value=0.0001	18540±10.40 C.V(%)=0.09 P-Value=0.0001	3994±9.248 C.V(%)=0.40 P-Value=0.0001	↑	↑	↓	↑	↑	↑
B-5	116±0.924 C.V(%)=1.37 P-Value=0.0001	128±0.635 C.V(%)=0.85 P-Value=0.0001	344±0.000 C.V(%)=0.000 P-Value=0.000	16±0.098 C.V(%)=1.06 P-Value=0.0001	8160±8.092 C.V(%)=0.17 P-Value=0.0001	2626±23.12 C.V(%)=1.52 P-Value=0.0001	↑	N	↓	N	↓	↓
B-6	24±0.144 C.V(%)=1.04 P-Value=0.0001	364±1.734 C.V(%)=0.82 P-Value=0.0001	282±3.468 C.V(%)=2.12 P-Value=0.0002	16±0.098 C.V(%)=1.06 P-Value=0.0001	12700±8.092 C.V(%)=0.11 P-Value=0.0001	2842±8.092 C.V(%)=0.49 P-Value=0.0001	↓	↑	↓	N	↓	↓
B-7	20±0.121 C.V(%)=1.05 P-Value=0.0001	124±0.693 C.V(%)=0.96 P-Value=0.0001	336±1.156 C.V(%)=0.59 P-Value=0.0001	14±0.080 C.V(%)=1.00 P-Value=0.0001	16780±30.05 C.V(%)=0.30 P-Value=0.0001	2806±18.49 C.V(%)=1.14 P-Value=0.0001	↓	↑	↓	↓	↑	↓
B-8	92±0.693 C.V(%)=1.30 P-Value=0.0001	150±0.924 C.V(%)=1.06 P-Value=0.0001	292±3.468 C.V(%)=2.05 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	18060±9.248 C.V(%)=0.08 P-Value=0.0001	3018±6.936 C.V(%)=0.39 P-Value=0.0001	↑	↑	↓	↓	↑	↑
C-9	112±0.693 C.V(%)=1.07 P-Value=0.0001	122±0.578 C.V(%)=0.81 P-Value=0.0001	216±2.890 C.V(%)=2.31 P-Value=0.0002	18±0.098 C.V(%)=0.94 P-Value=0.0001	14360±6.936 C.V(%)=0.08 P-Value=0.0001	2640±8.092 C.V(%)=0.53 P-Value=0.0001	↑	↓	↓	↑	↑	↓
C-10	20±0.115 C.V(%)=1.00 P-Value=0.0001	360±2.023 C.V(%)=0.97 P-Value=0.0001	300±4.046 C.V(%)=2.33 P-Value=0.0002	14±0.086 C.V(%)=1.07 P-Value=0.0001	10860±15.02 C.V(%)=0.23 P-Value=0.0001	2672±6.936 C.V(%)=0.449 P-Value=0.0001	↑	↑	↓	↓	↓	↓
C-11	18±0.098 C.V(%)=0.94 P-Value=0.0001	120±0.578 C.V(%)=0.83 P-Value=0.0001	346±4.624 C.V(%)=2.31 P-Value=0.0002	18±0.104 C.V(%)=1.00 P-Value=0.0001	14180±15.02 C.V(%)=0.18 P-Value=0.0001	2736±13.87 C.V(%)=0.87 P-Value=0.0001	↑	↓	↓	↑	↑	↓
C-12	26±0.115 C.V(%)=0.76 P-Value=0.0001	128±0.693 C.V(%)=0.93 P-Value=0.0001	734±3.468 C.V(%)=0.81 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	14120±12.71 C.V(%)=0.15 P-Value=0.0001	2852±10.40 C.V(%)=0.63 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.13
Sample.12. *Spinacea-oleracea* (Spinach)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



13. *Hibiscus-esculentus*. (Lady finger) local variety

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was $(36 \pm 0.127, 116 \pm 0.664, 54 \pm 0.462, 12 \pm 0.086, 3272 \pm 26.58$ and 294 ± 2.890 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe $(70 \pm 0.404, 152 \pm 1.156, 64 \pm 0.693, 18 \pm 0.115, 3758 \pm 12.71$ and $382 \pm 2.427)$ mg/Kg on dry weight basis in sub-boxes (A-1), (C-10), (A-4), (A-1), (A-4) and (A-4) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (34, 36, 10, 6, 486 and 88) mg/Kg dry weight basis. While in sub-boxes (B-7, C-10, C-11), (B-7), (B-5), (C-11), (B-6) and (B-5) minimum concentration of Cr, Zn, Mn, Cu, Mg and Fe $(34 \pm 0.115-0.144, 100 \pm 0.635, 26 \pm 0.121, 10 \pm 0.052, 3172 \pm 21.96$ and 212 ± 2.023 with total decrease of 2, 16, 28, 2, 100 and 82) mg/Kg dry weight basis was observed (Table 3.14 and Figure 3.14).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (A-1) at 1st stage of supplementation. Chromium content was decreased where magnesium and zinc were applied alone with no change in sub-box (B-6).

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (C-10) at 2nd stage of supplementation. Zinc content decreased where chromium was applied alone and no change occurred in sub-box (A-1). In case of magnesium there was decrease in one sub-box (B-7) and increase in other sub-box (C-11).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes except decrease in one sub-box (B-8). Marked increase occurred in sub-box (A-4) at 1st stage of supplementation. The concentration was increased in other sub-boxes supplemented with chromium and zinc with only exception of decrease in one sub-box (B-6).

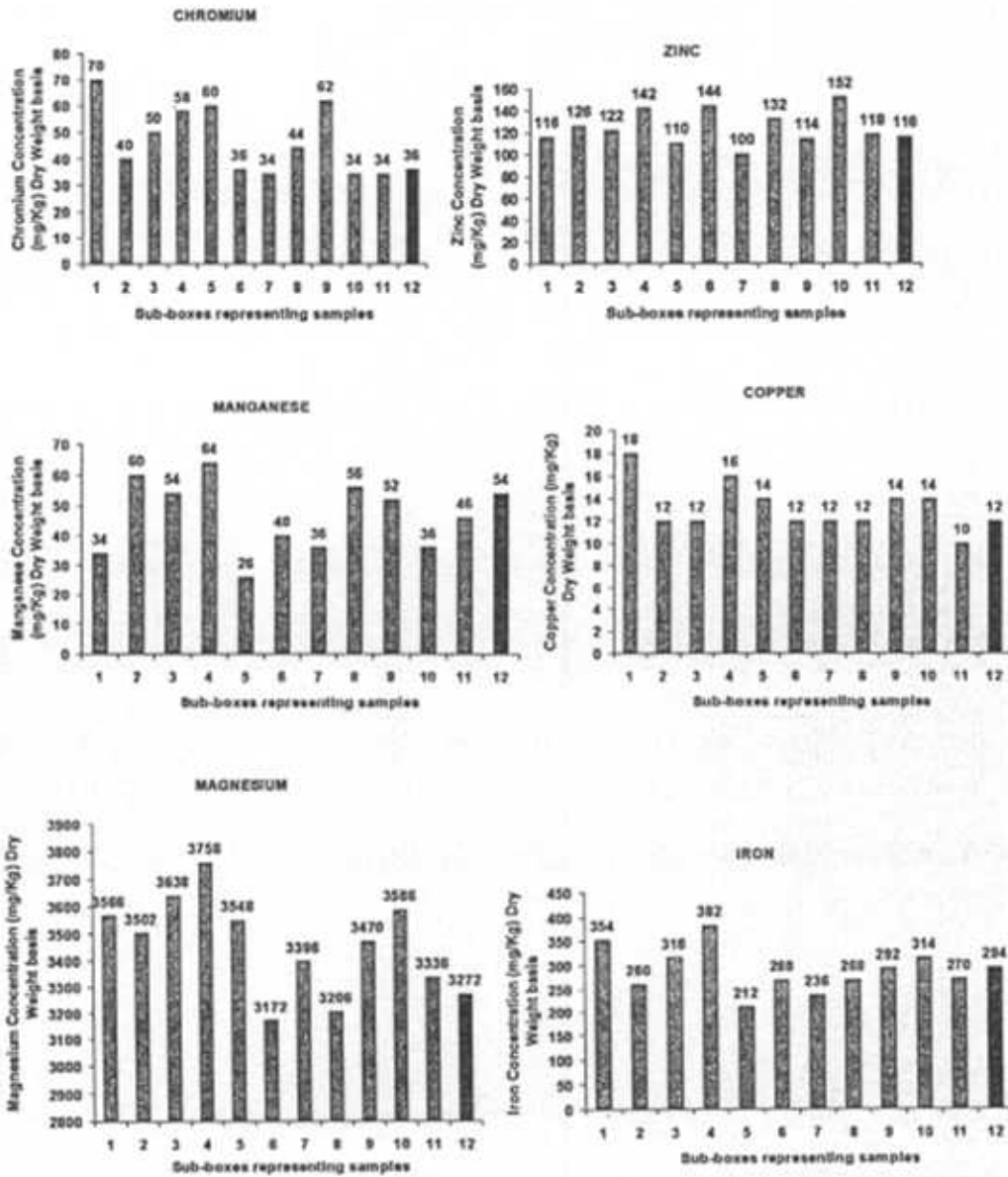
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper, manganese and iron was occurred in all sub-boxes with marked increase in sub-boxes (A-1), (A-4) and (A-4) respectively.

Table 3.14 *Hibiscus-esculentus*. (Lady finger) local variety Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	70±0.404 C.V(%)=1.00 P-Value=0.0001	116±0.664 C.V(%)=0.99 P-Value=0.0001	34±0.144 C.V(%)=0.73 P-Value=0.0001	18±0.115 C.V(%)=1.11 P-Value=0.0001	356±15.02 C.V(%)=0.72 P-Value=0.0001	354±1.734 C.V(%)=0.84 P-Value=0.0001	↑	N	↓	↑	↑	↑
A-2	40±0.173 C.V(%)=0.75 P-Value=0.0001	126±1.040 C.V(%)=1.42 P-Value=0.0001	60±0.635 C.V(%)=1.83 P-Value=0.0001	12±0.080 C.V(%)=1.16 P-Value=0.0001	350±2.10.40 C.V(%)=0.51 P-Value=0.0001	260±2.312 C.V(%)=1.53 P-Value=0.0001	↑	↑	↑	N	↑	↓
A-3	50±0.202 C.V(%)=0.70 P-Value=0.0001	122±0.924 C.V(%)=1.31 P-Value=0.0001	54±0.578 C.V(%)=1.85 P-Value=0.0001	12±0.080 C.V(%)=1.16 P-Value=0.0001	363±12.71 C.V(%)=0.60 P-Value=0.0001	316±3.468 C.V(%)=1.89 P-Value=0.0001	↑	↑	N	N	↑	↑
A-4	58±0.231 C.V(%)=0.68 P-Value=0.0001	142±0.924 C.V(%)=1.12 P-Value=0.0001	64±0.693 C.V(%)=1.87 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	375±12.71 C.V(%)=0.58 P-Value=0.0001	382±2.427 C.V(%)=1.09 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	60±0.231 C.V(%)=0.66 P-Value=0.0001	110±0.462 C.V(%)=0.72 P-Value=0.0001	26±0.121 C.V(%)=0.80 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	354±13.87 C.V(%)=0.67 P-Value=0.0001	212±2.023 C.V(%)=1.65 P-Value=0.0001	↑	↓	↓	↑	↑	↓
B-6	36±0.144 C.V(%)=0.69 P-Value=0.0001	144±0.635 C.V(%)=0.763 P-Value=0.0001	40±0.144 C.V(%)=0.62 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	317±21.96 C.V(%)=1.19 P-Value=0.0001	268±1.734 C.V(%)=1.11 P-Value=0.0001	N	↑	↓	N	↓	↓
B-7	34±0.115 C.V(%)=0.58 P-Value=0.0001	100±0.635 C.V(%)=1.10 P-Value=0.0001	36±0.115 C.V(%)=0.55 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	339±11.56 C.V(%)=0.58 P-Value=0.0001	236±1.502 C.V(%)=1.10 P-Value=0.0001	↓	↓	↓	N	↑	↓
B-8	44±0.121 C.V(%)=0.47 P-Value=0.0001	132±0.809 C.V(%)=1.06 P-Value=0.0001	56±0.462 C.V(%)=1.42 P-Value=0.0001	12±0.080 C.V(%)=1.16 P-Value=0.0001	320±33.52 C.V(%)=1.80 P-Value=0.0001	268±2.196 C.V(%)=1.41 P-Value=0.0001	↑	↑	↑	N	↓	↓
C-9	62±0.121 C.V(%)=0.33 P-Value=0.0001	114±0.693 C.V(%)=1.05 P-Value=0.0001	52±0.462 C.V(%)=1.53 P-Value=0.0001	14±0.092 C.V(%)=1.14 P-Value=0.0001	3470±9.248 C.V(%)=0.46 P-Value=0.0001	292±2.601 C.V(%)=1.54 P-Value=0.0001	↑	↓	↓	↑	↑	↓
C-10	34±0.115 C.V(%)=0.58 P-Value=0.0001	152±1.156 C.V(%)=1.31 P-Value=0.0001	36±0.121 C.V(%)=0.58 P-Value=0.0001	14±0.080 C.V(%)=1.00 P-Value=0.0001	358±12.71 C.V(%)=0.61 P-Value=0.0001	314±2.312 C.V(%)=1.27 P-Value=0.0001	↓	↑	↓	↑	↑	↑
C-11	34±0.144 C.V(%)=0.73 P-Value=0.0001	118±0.664 C.V(%)=0.97 P-Value=0.0001	46±0.144 C.V(%)=0.54 P-Value=0.0001	10±0.052 C.V(%)=0.90 P-Value=0.0001	333±11.56 C.V(%)=0.59 P-Value=0.0001	270±1.734 C.V(%)=1.11 P-Value=0.0001	↓	↑	↓	↓	↑	↓
C-12	36±0.127 C.V(%)=0.61 P-Value=0.0001	116±0.664 C.V(%)=0.99 P-Value=0.0001	54±0.462 C.V(%)=1.48 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	327±26.58 C.V(%)=1.40 P-Value=0.0001	294±2.890 C.V(%)=1.70 P-Value=0.0001	C	C	C	C	C	C

N= No Increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.14
Sample 13. *Hibiscus-esculentus* (Ladyfinger Local)
Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



14. *Hibiscus-esculentus*. (Lady finger) Indian variety

The concentration of Cr, Zn, Mn, Cu, Mg and Fe in control samples observed was $(40 \pm 0.115, 106 \pm 0.404, 64 \pm 0.404, 14 \pm 0.086, 2876 \pm 12.71$ and 356 ± 1.156 with a maximum concentration of Cr, Zn, Mn, Cu, Mg and Fe ($64 \pm 0.693, 216 \pm 1.156, 78 \pm 0.635, 20 \pm 0.115, 3484 \pm 6.936$ and 376 ± 1.849) mg/Kg on dry weight basis in sub-boxes (C-9), (C-9),(A-4),(A-4), (A-2) and (A-3) respectively. The total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (24, 110, 14, 6, 608 and 20) mg/Kg dry weight basis. While in sub-boxes (C-10), (B-5), (A-3), (A-3,B-5,C-9) and (A-1) minimum concentration of Cr, Zn, Mn, Cu and Fe ($36 \pm 0.115, 100 \pm 0.664, 36 \pm 0.121, 12 \pm 0.080-0.086$ and 188 ± 1.734 with total decrease of 4, 6, 28, 2 and 168) mg/Kg dry weight basis was observed (Table 3.15 and Figure 3.15).

Effect on Cr, Zn, Mn, Cu, Mg and Fe Uptake Supplied in Combination or Individually and also Stage of Application

Chromium was applied in the form of combinations (1, 2, 3, 4, 8) and individually (5, 9). Increase in chromium content was occurred in all sub-boxes with marked increase in sub-box (C-9) at 2nd stage of supplementation. Chromium content remained unchanged where zinc and magnesium were applied in sub-boxes (B-6) and (B-7), while decreased in sub-boxes (C-10) and (C-11) respectively.

Zinc was applied in the form of combinations (2, 3, 4, 8) and individually (6, 10). Zinc content was increased in all sub-boxes with marked increase in sub-box (C-9) at 2nd stage of supplementation. Zinc content decreased at 1st stage and increased at 2nd stage in chromium supplemented sub-boxes (B-5) and (C-9) respectively. Similarly, magnesium application at 1st stage in sub-box (B-7) caused increase and no change at 2nd stage in sub-box (C-11) of zinc content. Decrease also occurred in sub-box (A-1).

Magnesium was applied in the form of combinations (1, 4, 8) and individually (7, 11). Magnesium concentration was increased in all sub-boxes with marked increase in sub-box (A-2) at 1st stage of supplementation where no magnesium was supplemented. The concentration was increased in sub-boxes supplemented with chromium and zinc.

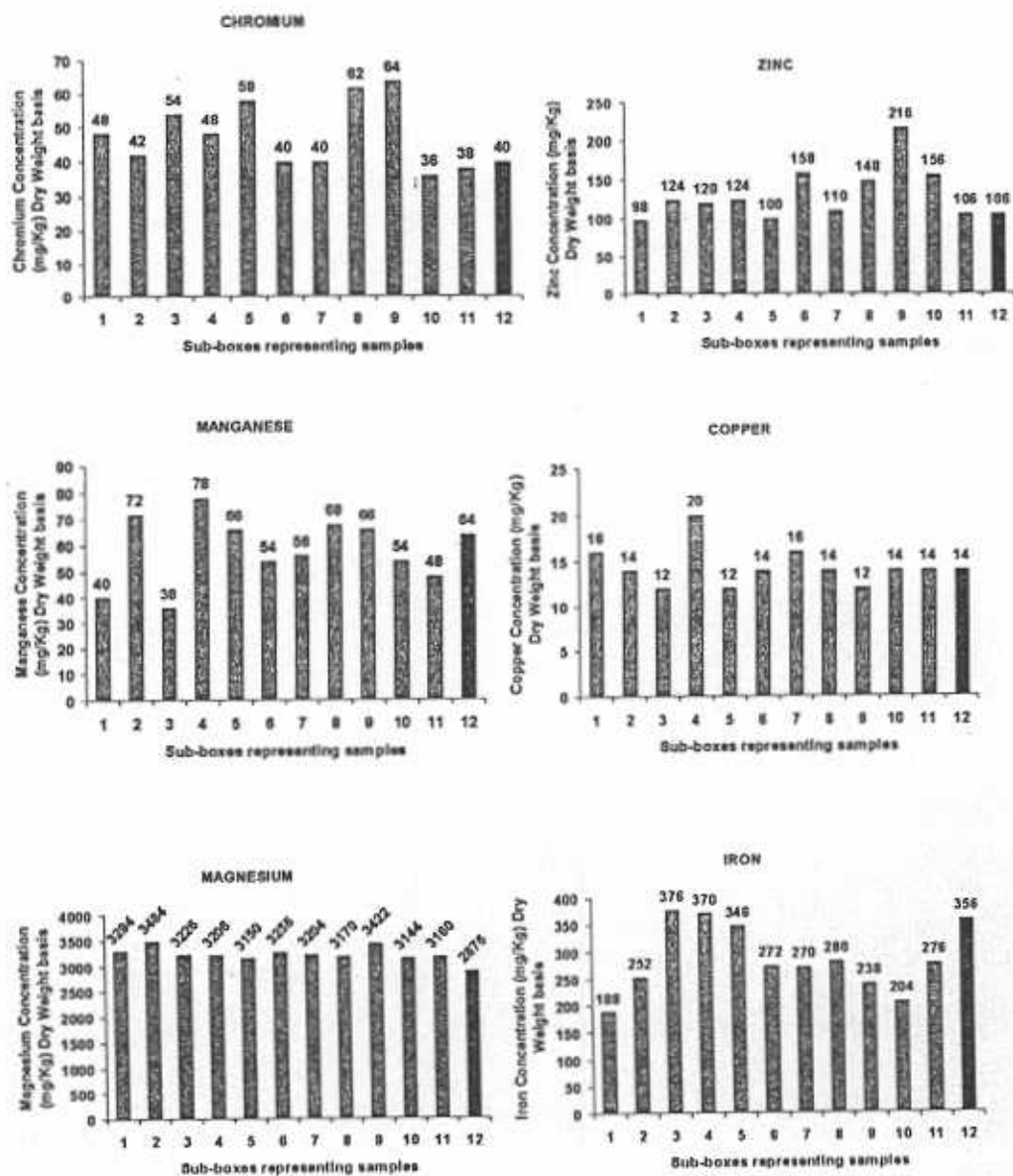
Copper, manganese and iron were applied in combinations (1, 4), (2, 4) and (3, 4) respectively. Increase in concentration of copper, manganese and iron was occurred in all sub-boxes with marked increase in sub-boxes (A-4), (A-4) and (A-3) respectively.

Table 3.15 *Hibiscus-esculentus*. (Lady finger) Indian variety Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) Dry Weight Basis and Effect on their Uptake When Supplemented Individually or in Combination

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis						Effect on their Uptake When Supplemented Individually or in Combination Decrease(↓) Increase(↑)					
	Cr	Zn	Mn	Cu	Mg	Fe	Cr	Zn	Mn	Cu	Mg	Fe
A-1	48±0.173 C.V(%)=0.62 P-Value=0.0001	98±0.578 C.V(%)=1.02 P-Value=0.0001	40±0.144 C.V(%)=0.62 P-Value=0.0001	16±0.098 C.V(%)=1.06 P-Value=0.0001	3294±10.40 C.V(%)=0.54 P-Value=0.0001	188±1.734 C.V(%)=1.59 P-Value=0.0001	↑	↑	↑	↑	↑	↑
A-2	42±0.144 C.V(%)=0.59 P-Value=0.0001	124±0.635 C.V(%)=0.88 P-Value=0.0001	72±0.462 C.V(%)=1.11 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	3484±6.936 C.V(%)=0.34 P-Value=0.0001	252±1.618 C.V(%)=1.11 P-Value=0.0001	↑	↑	↑	N	↑	↑
A-3	54±0.202 C.V(%)=0.64 P-Value=0.0001	120±0.924 C.V(%)=1.33 P-Value=0.0001	36±0.121 C.V(%)=0.58 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	3226±8.092 C.V(%)=0.43 P-Value=0.0001	376±1.849 C.V(%)=0.85 P-Value=0.0001	↑	↑	↑	↓	↑	↑
A-4	48±0.462 C.V(%)=1.66 P-Value=0.0001	124±1.040 C.V(%)=1.45 P-Value=0.0001	78±0.635 C.V(%)=1.41 P-Value=0.0001	20±0.115 C.V(%)=1.00 P-Value=0.0001	3206±5.780 C.V(%)=0.31 P-Value=0.0001	370±2.312 C.V(%)=1.08 P-Value=0.0001	↑	↑	↑	↑	↑	↑
B-5	58±0.693 C.V(%)=2.06 P-Value=0.0001	100±0.664 C.V(%)=1.15 P-Value=0.0001	66±0.693 C.V(%)=1.81 P-Value=0.0001	12±0.080 C.V(%)=1.16 P-Value=0.0001	3150±16.18 C.V(%)=0.88 P-Value=0.0001	346±2.890 C.V(%)=1.44 P-Value=0.0001	↑	↓	↑	↓	↑	↓
B-6	40±0.144 C.V(%)=0.62 P-Value=0.0001	158±1.156 C.V(%)=1.26 P-Value=0.0001	54±0.404 C.V(%)=1.29 P-Value=0.0001	14±0.080 C.V(%)=1.00 P-Value=0.0001	3258±5.780 C.V(%)=0.30 P-Value=0.0001	272±2.023 C.V(%)=1.28 P-Value=0.0001	N	↑	↓	N	↑	↓
B-7	40±0.144 C.V(%)=0.62 P-Value=0.0001	110±0.462 C.V(%)=0.72 P-Value=0.0001	56±0.404 C.V(%)=1.25 P-Value=0.0001	16±0.086 C.V(%)=0.93 P-Value=0.0001	3204±8.092 C.V(%)=0.43 P-Value=0.0001	270±1.445 C.V(%)=0.92 P-Value=0.0001	N	↑	↓	↑	↑	↓
B-8	62±0.578 C.V(%)=1.61 P-Value=0.0001	148±0.693 C.V(%)=0.81 P-Value=0.0001	68±0.462 C.V(%)=1.17 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	3170±24.27 C.V(%)=1.32 P-Value=0.0001	280±1.271 C.V(%)=0.785 P-Value=0.0001	↑	↑	↑	N	↑	↓
C-9	64±0.693 C.V(%)=1.87 P-Value=0.0001	216±1.156 C.V(%)=0.92 P-Value=0.0001	66±0.462 C.V(%)=1.21 P-Value=0.0001	12±0.086 C.V(%)=1.25 P-Value=0.0001	3422±10.40 C.V(%)=0.52 P-Value=0.0001	238±0.693 C.V(%)=0.50 P-Value=0.0001	↑	↑	↑	↑	↑	↓
C-10	36±0.115 C.V(%)=0.55 P-Value=0.0001	156±1.040 C.V(%)=1.15 P-Value=0.0001	54±0.462 C.V(%)=1.481 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	3144±15.02 C.V(%)=0.82 P-Value=0.0001	204±1.734 C.V(%)=1.47 P-Value=0.0001	↓	↑	↓	N	↑	↓
C-11	38±0.121 C.V(%)=0.55 P-Value=0.0001	106±0.404 C.V(%)=0.66 P-Value=0.0001	48±0.202 C.V(%)=0.72 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	3160±6.936 C.V(%)=0.37 P-Value=0.0001	276±2.080 C.V(%)=1.30 P-Value=0.0001	↓	N	↓	N	↑	↓
C-12	40±0.115 C.V(%)=0.50 P-Value=0.0001	106±0.404 C.V(%)=0.66 P-Value=0.0001	64±0.404 C.V(%)=1.09 P-Value=0.0001	14±0.086 C.V(%)=1.07 P-Value=0.0001	2876±12.71 C.V(%)=0.76 P-Value=0.0001	356±1.156 C.V(%)=0.56 P-Value=0.0001	C	C	C	C	C	C

N= No increase/decrease, C= Control, Mean±S.E (Standard error)

Figure 3.15
 Sample.14. *Hibiscus-esculentus*. (Ladyfinger Indian)
 Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg Dry Weight Basis



WINTER VEGETABLE SAMPLES WITH MAXIMUM Cr, Zn, Mn, Cu, Mg and Fe CONCENTRATION

In winter vegetables maximum concentration of chromium in milligram per Kilogram dry weight basis has been shown in the order (*Portulaca-oleracea* 54>*Spinacea-oleracea* 42> *Trigonella-foenum-graceum* 34> *Brassica-rapa* Roots and Leaves 24> *Allium-sativum* 22> *Brassica-campestris-var-sarson* 20> *Raphanus-sativus* Leaves 16> *Raphanus-sativus* Roots 14 (Table 1a and Figure 1a-1). While chromium content in milligram per Kilogram dry weight basis in the control samples was (*Portulaca-oleracea* 14, *Spinacea-oleracea* 22, *Trigonella-foenum-graceum* 24, *Brassica-rapa* Roots 6 and Leaves 8, *Allium-sativum* 8, *Brassica-campestris-var-sarson* 8, *Raphanus-sativus* Leaves 10 and *Raphanus-sativus* Roots 8.

In winter vegetables maximum concentration of zinc in milligram per Kilogram dry weight basis was observed in the order of *Portulaca-oleracea* 316> *Spinacea-oleracea* 196> *Raphanus-sativus* Leaves 166> *Trigonella-foenum-graceum* 162> *Brassica-rapa* Leaves 152> *Brassica-rapa* Roots 146> *Brassica-campestris-var-sarson* 122> *Raphanus-sativus* Roots 120> *Allium-sativum* 120 (Table 1a and Figure 1a-2). While zinc content in milligram per Kilogram dry weight basis of the control samples was *Portulaca-oleracea* 88, *Spinacea-oleracea* 13, *Raphanus-sativus* Leaves 122, *Trigonella-foenum-graceum* 68, *Brassica-rapa* Leaves 88, *Brassica-rapa* Roots 78, *Brassica-campestris-var-sarson* 92, *Raphanus-sativus* Roots 58 and *Allium-sativum* 92.

In winter vegetables maximum concentration of manganese in milligram per Kilogram dry weight basis was observed in the order of *Spinacea-oleracea* 196> *Brassica-campestris-var-sarson* 164> *Portulaca-oleracea* 110> *Trigonella-foenum-graceum* 76> *Allium-sativum* 24 (Table 1a and Figure 1a-3). While manganese content in milligram per Kilogram dry weight basis in control samples was *Allium-sativum* 20, *Portulaca-oleracea* 90, *Brassica-campestris-var-sarson* 142, *Trigonella-foenum-graceum* 66 and *Spinacea-oleracea* 164.

In winter vegetables maximum concentration of copper in milligram per Kilogram dry weight basis was observed in the order of *Raphanus-sativus* Leaves 44> *Raphanus-sativus* Roots 30> *Portulaca-oleracea* 22> *Brassica-rapa* Leaves 18> *Allium-sativum* 14> *Brassica-rapa* Roots 12 (Table 1a and Figure 1a-4). While copper content in milligram per Kilogram dry

weight basis in control samples was *Raphanus-sativus* Leaves 12, *Raphanus-sativus* Roots 6, *Portulaca-oleracea* 14, *Brassica-rapa* Leaves 12, *Allium-sativum* 10 and *Brassica-rapa* Roots 8.

In winter vegetables maximum concentration of magnesium in milligram per Kilogram dry weight basis was observed in the order of *Portulaca-oleracea* 23120> *Spinacea-oleracea* 16460> *Brassica-campestris-var-sarson* 4154> *Trigonella-foenum-graceum* 3264> *Raphanus-sativus* Roots 2476> *Allium-sativum* 902 (Table 1a and Figure 1a-5). While magnesium content in milligram per Kilogram dry weight basis in the control samples was *Portulaca-oleracea* 13660, *Spinacea-oleracea* 14740, *Brassica-campestris-var-sarson* 3730, *Trigonella-foenum-graceum* 3008, *Raphanus-sativus* Roots 2264 and *Allium-sativum* 786.

In winter vegetables maximum concentration of iron in milligram per Kilogram dry weight basis was observed in the order of *Portulaca-oleracea* 3548> *Spinacea-oleracea* 3498> *Trigonella-foenum-graceum* 2092> *Brassica-rapa* Leaves 1926> *Brassica-rapa* Roots 808> *Allium-sativum* 158 (Table 1a and Figure 1a-6). While iron content in milligram per Kilogram dry weight basis in the control samples was *Portulaca-oleracea* 2864, *Spinacea-oleracea* 3124, *Trigonella-foenum-graceum* 1572, *Brassica-rapa* Leaves 1854, *Brassica-rapa* Roots 620 and *Allium-sativum* 150.

Table 1 a
Winter Vegetable Samples with Maximum Cr, Zn, Mn, Cu, Mg and Fe Concentration

a. Samples with Maximum Chromium Concentration				
S.No	Samples	Sub-box with Maximum Concentration	Supplementation Stage	Maximum Concentration (mg/Kg) Dry Weight Basis
1	<i>Portulaca-oleracea</i> (Pot purslane)	(8)	1 st	54
2	<i>Spinacea-oleracea</i> (Spinach)	(5)	1 st	42
3	<i>Trigonella-foenum-graceum</i> (Fenugreek/ Methi)	(5)	1 st	34
4	<i>Brassica-rapa</i> (Turnip Roots)	(5)	1 st	24
5	<i>Brassica-rapa</i> (Turnip Leaves)	(9)	2 nd	24
6	<i>Allium-sativum</i> (Garlic)	(5)	1 st	22
7	<i>Brassica-campestris-var-sarson</i> (Sarson)	(5)	1 st	20
8	<i>Raphanus-sativus</i> (Radish Leaves)	(4,5,9)	1 st , 2 nd	16
9	<i>Raphanus-sativus</i> (Radish Roots)	(9)	2 nd	14
b. Samples with Maximum Zinc Concentration				
1	<i>Portulaca-oleracea</i> (Pot purslane)	(8)	1 st	316
2	<i>Spinacea-oleracea</i> (Spinach)	(4)	1 st	196
3	<i>Raphanus-sativus</i> (Radish Leaves)	(6)	1 st	166
4	<i>Trigonella-foenum-graceum</i> (Fenugreek/ Methi)	(4)	1 st	162
5	<i>Brassica-rapa</i> (Turnip Leaves)	(6)	1 st	152
6	<i>Brassica-rapa</i> (Turnip Roots)	(2)	1 st	146
7	<i>Brassica-campestris-var-sarson</i> (Sarson)	(3,6)	1 st	122
8	<i>Raphanus-sativus</i> (Radish Roots)	(6)	1 st	120
9	<i>Allium-sativum</i> (Garlic)	(6)	1 st	120
c. Samples with Maximum Manganese Concentration				
1	<i>Spinacea-oleracea</i> (Spinach)	(4)	1 st	196
2	<i>Brassica-campestris-var-sarson</i> (Sarson)	(4)	1 st	164
3	<i>Portulaca-oleracea</i> (Pot purslane)	(4)	1 st	110
4	<i>Trigonella-foenum-graceum</i> (Fenugreek/Methi)	(4)	1 st	76
5	<i>Allium-sativum</i> (Garlic)	(4)	1 st	24
d. Samples with Maximum Copper Concentration				
1	<i>Raphanus-sativus</i> (Radish Leaves)	(1)	1 st	44
2	<i>Raphanus-sativus</i> (Radish Roots)	(1)	1 st	30
3	<i>Portulaca-oleracea</i> (Pot purslane)	(1)	1 st	22
4	<i>Brassica-rapa</i> (Turnip Leaves)	(4)	1 st	18
5	<i>Allium-sativum</i> (Garlic)	(1, 4, 5, 6)	1 st	14
6	<i>Brassica-rapa</i> (Turnip Roots)	(1)	1 st	12
e. Samples with Maximum Magnesium Concentration				
1	<i>Portulaca-oleracea</i> (Pot purslane)	(6)	1 st	23120
2	<i>Spinacea-oleracea</i> (Spinach)	(9)	2 nd	16460
3	<i>Brassica-campestris-var-sarson</i> (Sarson)	(1)	1 st	4154
4	<i>Trigonella-foenum-graceum</i> (Fenugreek/Methi)	(7)	1 st	3264
5	<i>Raphanus-sativus</i> (Radish Roots)	(2)	1 st	2476
6	<i>Allium-sativum</i> (Garlic)	(10)	2 nd	902
f. Samples with Maximum Iron Concentration				
1	<i>Portulaca-oleracea</i> (Pot purslane)	(4)	1 st	3548
2	<i>Spinacea-oleracea</i> (Spinach)	(5)	1 st	3498
3	<i>Trigonella-foenum-graceum</i> (Fenugreek/Methi)	(3)	1 st	2092
4	<i>Brassica-rapa</i> (Turnip Leaves)	(5)	1 st	1926
5	<i>Brassica-rapa</i> (Turnip Roots)	(9)	2 nd	808
6	<i>Allium-sativum</i> (garlic)	(2)	1 st	158

Figure 1a
Winter Vegetables with Maximum Concentration of Cr, Zn, Mn, Cu, Mg and Fe
mg/Kg Dry Weight Basis

Figure 1a-1

Winter Vegetables with Maximum Chromium Concentration (mg/Kg) Dry Weight Basis

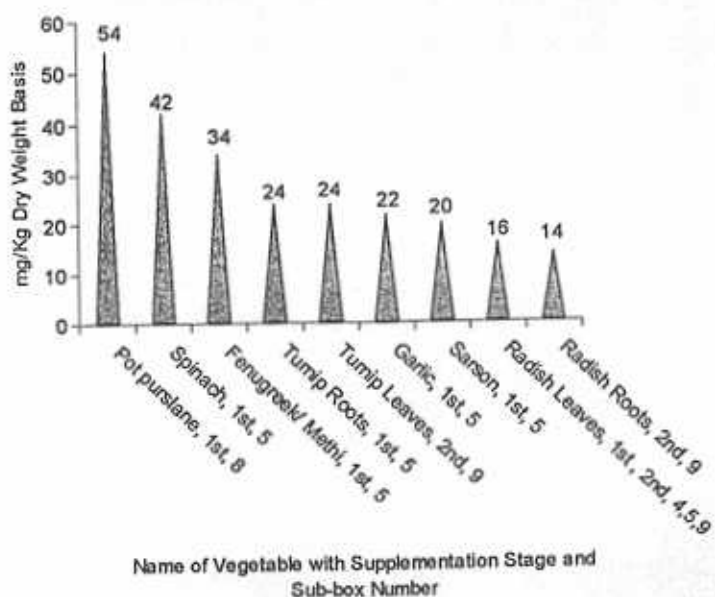


Figure 1a-2

Winter Vegetables with Maximum Zinc Concentration (mg/Kg) Dry Weight Basis

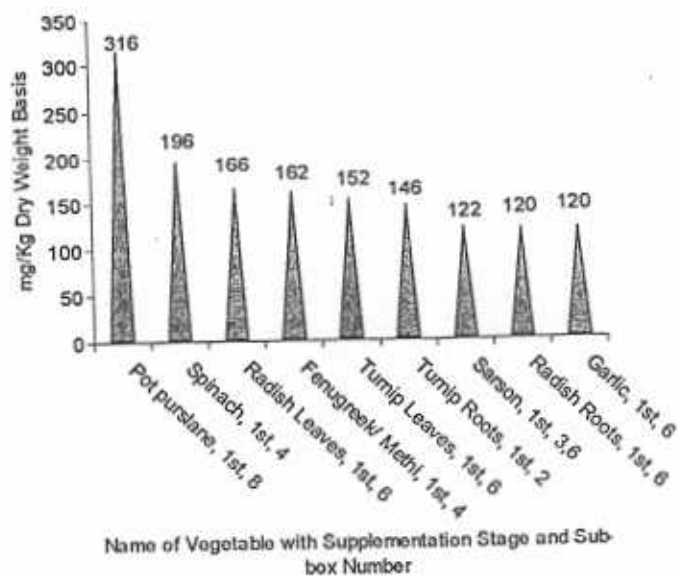


Figure 1a Continue.....

Figure 1a-3

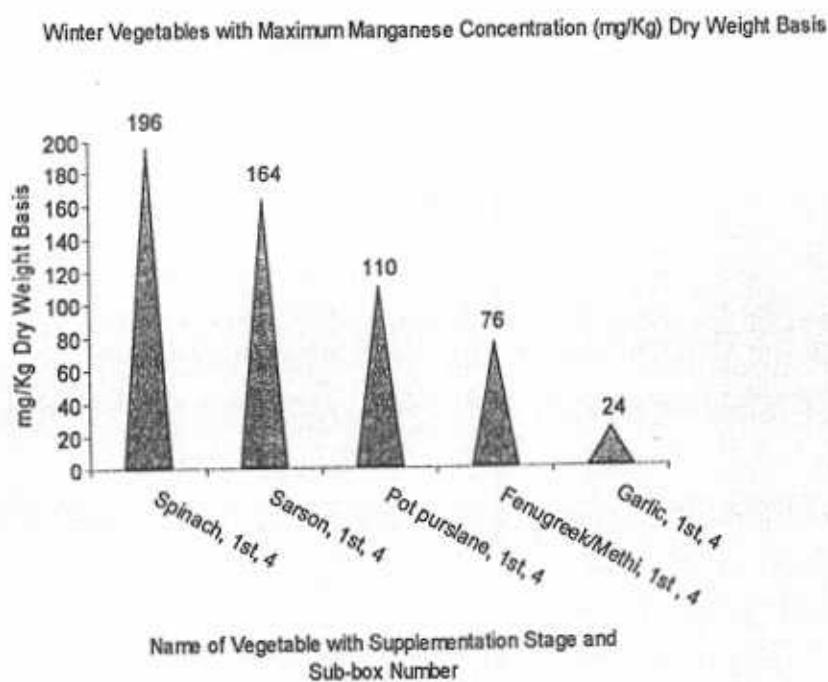


Figure 1a-4

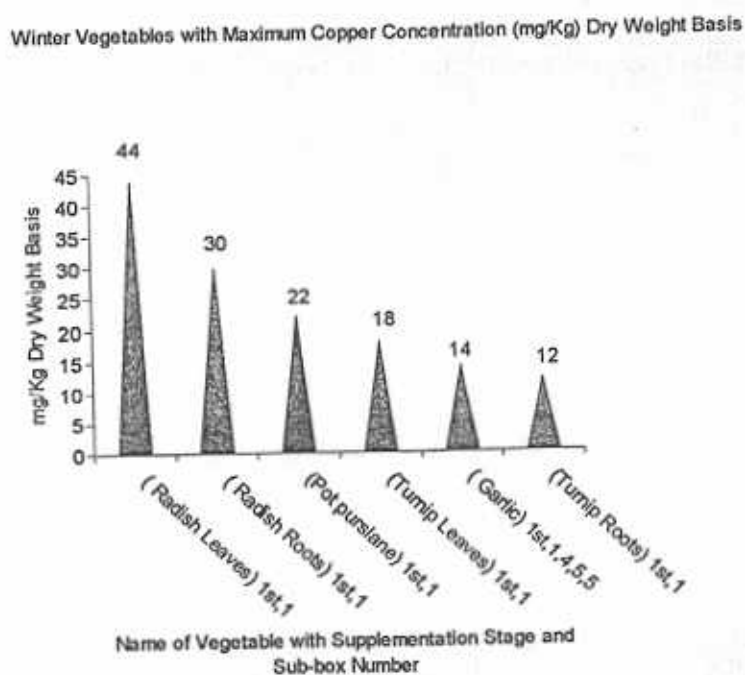


Figure 1a Continue.....

Figure 1a-5

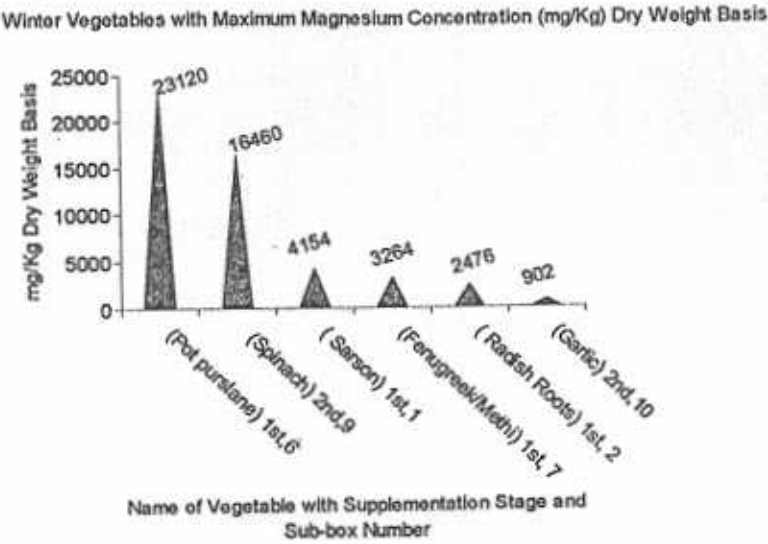
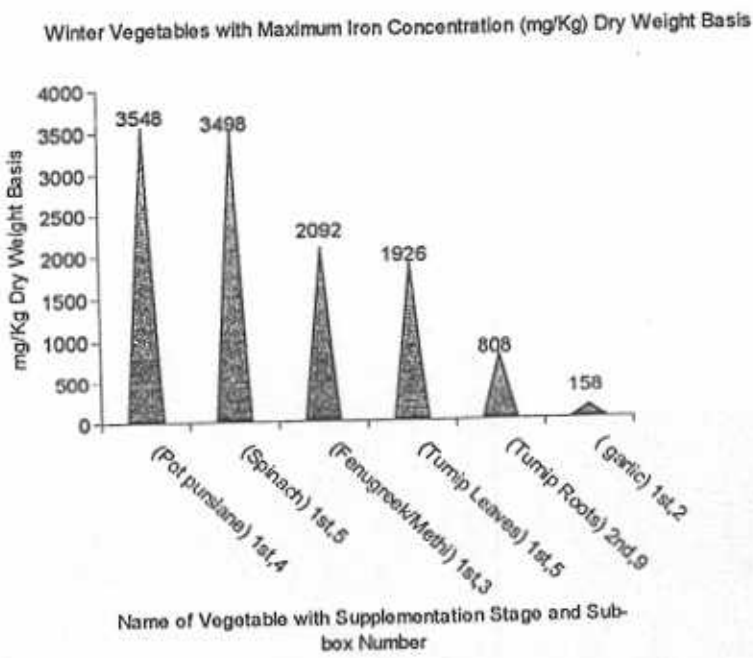


Figure 1a-6



WINTER VEGETABLE SAMPLES WITH MAXIMUM INCREASE of Cr, Zn, Mn, Cu, Mg and Fe

In winter vegetables total increase in chromium concentration milligram per Kilogram dry weight basis has been shown in the order (*Portulaca-oleracea* 40> *Spinacea-oleracea* 20> *Brassica-rapa* Roots 18>*Brassica-rapa* Leaves16 > *Allium-sativum* 14 > *Brassica-campestris-var-sarson* 12> *Trigonella-foenum-graceum*10 > *Raphanus-sativus* Leaves and Roots 6) (Table 1b and Figure 1b-1).

Vegetables with total increase in zinc concentration milligram per Kilogram dry weight basis observed in the order of *Portulaca-oleracea* 228> *Trigonella-foenum-graceum* 94> *Brassica-rapa* Roots 68> *Spinacea-oleracea* 66> *Brassica-rapa* Leaves 64> *Raphanus-sativus* Roots 62> *Raphanus-sativus* Leaves 44> *Brassica-campestris-var-sarson* 30> *Allium-sativum* 28 (Table 1b and Figure 1b-2).

Vegetables with total increase in manganese concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 32> *Brassica-campestris-var-sarson* 22> *Portulaca-olerace* 20> *Trigonella-foenum-graceum* 10> *Allium-sativum* 4 (Table 1b and Figure 1b-3).

Vegetables with total increase in copper concentration milligram per Kilogram dry weight basis were in the order of *Raphanus-sativus* Leaves 32> *Raphanus-sativus* Roots 24> *Portulaca-oleracea* 8> *Brassica-rapa* Leaves 6> *Brassica-rapa* Roots and *Allium-sativum* 4 (Table 1b and Figure 1b-4).

Vegetables with total increase in magnesium concentration milligram per Kilogram dry weight basis observed were in the order of *Portulaca-oleracea* 9460> *Spinacea-oleracea* 1720> *Brassica-campestris-var-sarson* 424> *Trigonella-foenum-graceum* 256> *Raphanus-sativus* Roots 212> *Allium-sativum* 116 (Table 1b and Figure 1b-5).

Vegetables with total increase in iron concentration milligram per Kilogram dry weight basis observed were in the order of *Portulaca-oleracea*684> *Trigonella-foenum-graceum* 520> *Spinacea-oleracea* 374> *Brassica-rapa* Roots 188> *Brassica-rapa* Leaves 72> *Allium-sativum* 8 (Table 1b and Figure 1b-6).

Table 1 b
Winter Vegetable Samples with Maximum Increase of Cr, Zn, Mn, Cu, Mg and Fe

a. Samples with Maximum Chromium Increase		
S.No	Samples	(mg/Kg) Dry Weight Basis
1	<i>Portulaca-oleracea</i> (Pot purslane)	40
2	<i>Spinacea-oleracea</i> (Spinach)	20
3	<i>Brassica-rapa</i> (Turnip Roots)	18
4	<i>Brassica-rapa</i> (Turnip Leaves)	16
5	<i>Allium-sativum</i> (Garlic)	14
6	<i>Brassica-campestris-var-sarson.</i> (Sarson)	12
7	<i>Trigonella-foenum-graceum.</i> (Fenugreek/ Methi)	10
8	<i>Raphanus-sativus</i> (Radish Leaves)	6
9	<i>Raphanus-sativus</i> (Radish Roots)	6
b. Samples with Maximum Zinc Increase		
1	<i>Portulaca-oleracea</i> (Pot purslane)	228
2	<i>Trigonella-foenum-graceum.</i> (Fenugreek/Methi)	94
3	<i>Brassica-rapa</i> (Turnip Roots)	68
4	<i>Spinacea-oleracea</i> (Spinach)	66
5	<i>Brassica-rapa</i> (Turnip Leaves)	64
6	<i>Raphanus-sativus</i> (Radish Roots)	62
7	<i>Raphanus-sativus</i> (Radish Leaves)	44
8	<i>Brassica-campestris-var-sarson.</i> (Sarson)	30
9	<i>Allium-sativum</i> (Garlic)	28
c. Samples with Maximum Manganese Increase		
1	<i>Spinacea-oleracea</i> (Spinach)	32
2	<i>Brassica-campestris-var-sarson.</i> (Sarson)	22
3	<i>Portulaca-oleracea</i> (Pot purslane)	20
4	<i>Trigonella-foenum-graceum</i> (Fenugreek/ Methi)	10
5	<i>Allium-sativum</i> (Garlic)	4
d. Samples with Maximum Copper Increase		
1	<i>Raphanus-sativus</i> (Radish Leaves)	32
2	<i>Raphanus-sativus</i> (Radish Roots)	24
3	<i>Portulaca-oleracea</i> (Pot purslane)	8
4	<i>Brassica-rapa</i> (Turnip Leaves)	6
5	<i>Brassica-rapa</i> (Turnip Roots)	4
6	<i>Allium-sativum</i> (Garlic)	4
e. Samples with Maximum Magnesium Increase		
1	<i>Portulaca-oleracea</i> (Pot purslane)	9460
2	<i>Spinacea-oleracea</i> (Spinach)	1720
3	<i>Brassica-campestris-var-sarson.</i> (Sarson)	424
4	<i>Trigonella-foenum-graceum.</i> (Fenugreek/Methi)	256
5	<i>Raphanus-sativus</i> (Radish Roots)	212
6	<i>Allium-sativum</i> (Garlic)	116
f. Samples with Maximum Iron Increase		
1	<i>Portulaca-oleracea</i> (Pot purslane)	684
2	<i>Trigonella-foenum-graceum</i> (Fenugreek/Methi)	520
3	<i>Spinacea-oleracea</i> (Spinach)	374
4	<i>Brassica-rapa</i> (Turnip Roots)	188
5	<i>Brassica-rapa</i> (Turnip Leaves)	72
6	<i>Allium-sativum</i> (Garlic)	8

Figure 1b
Winter Vegetables with Increase in Concentration of Cr, Zn, Mn, Cu, Mg and Fe
mg/Kg Dry Weight Basis

Figure 1b-1

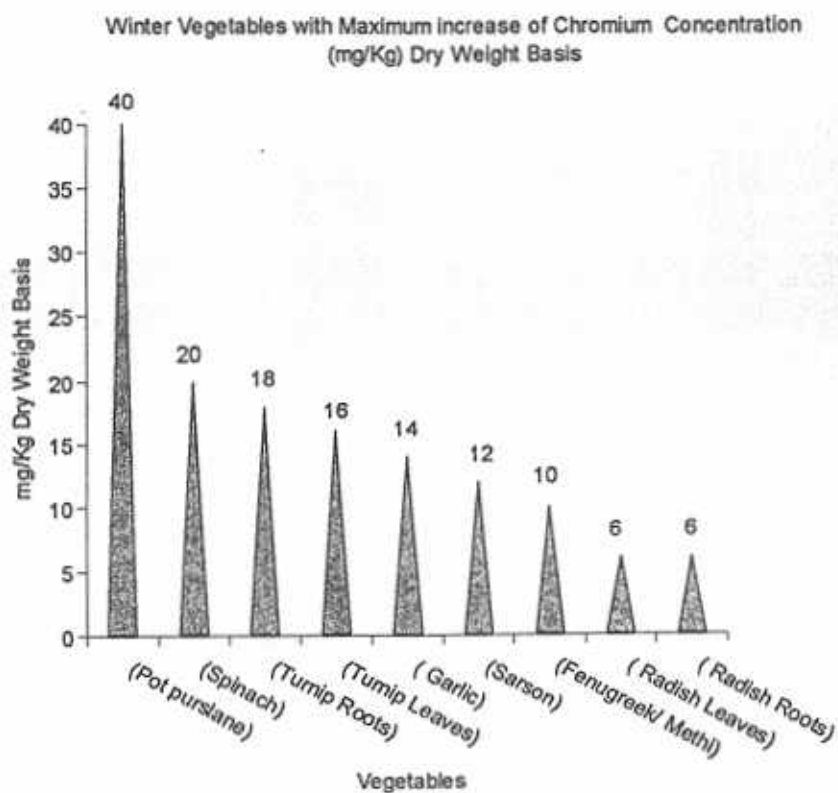


Figure 1b-2

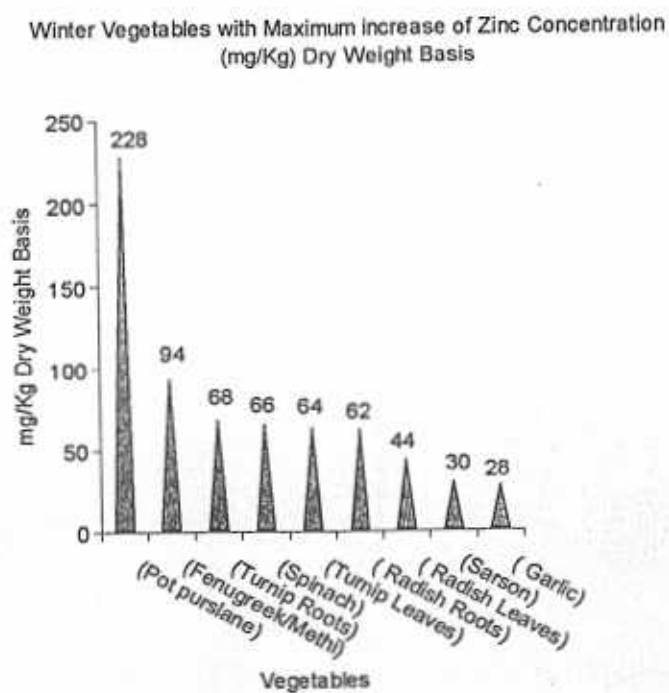


Figure 1b Continue.....

Figure 1b-3

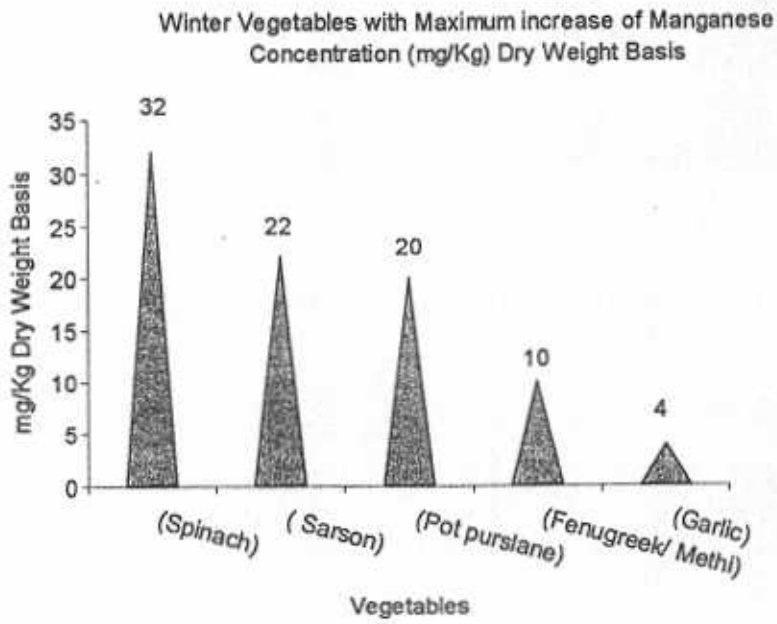


Figure 1b-4

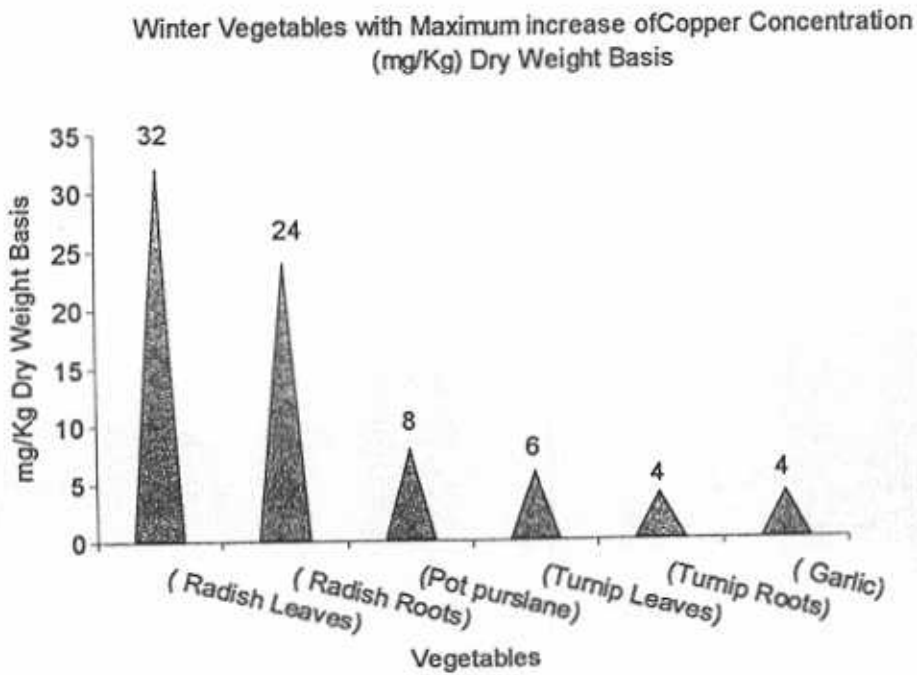


Figure 1b Continue.....

Figure 1b-5

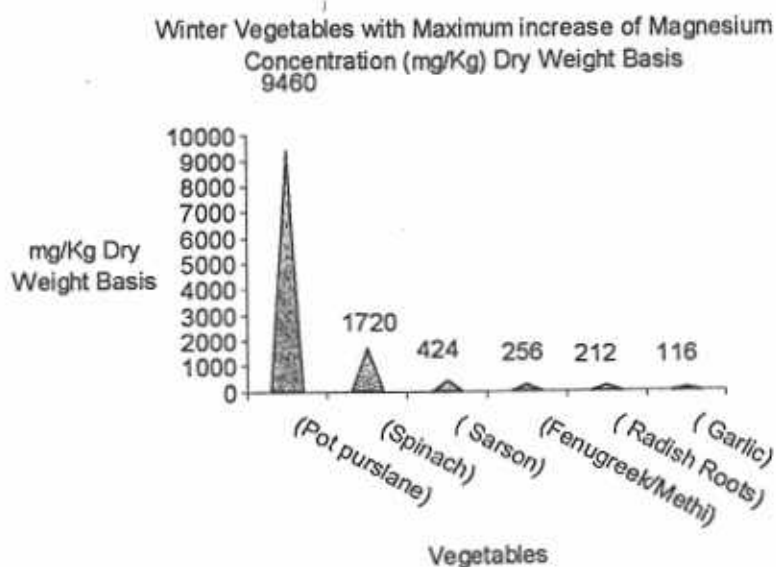
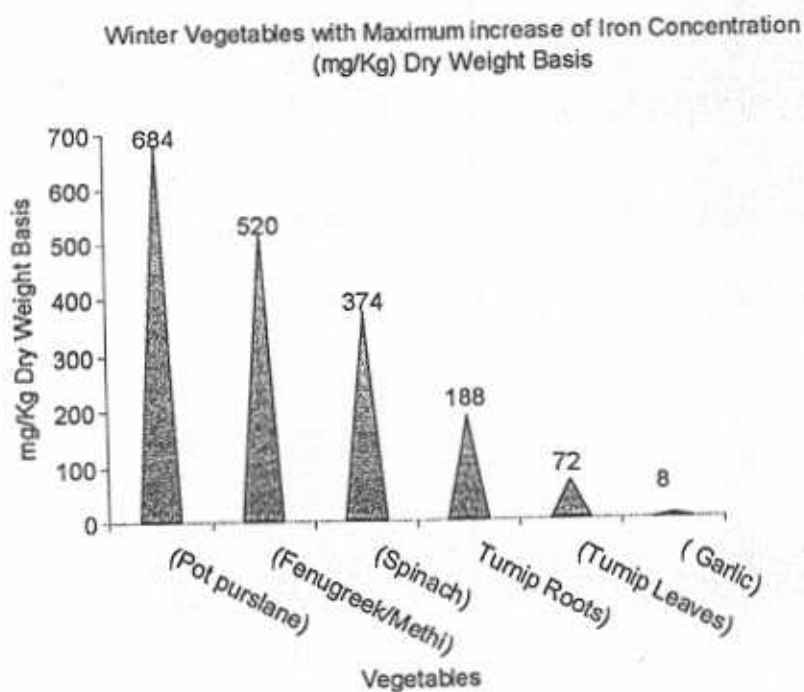


Figure 1b-6



SUMMER VEGETABLE SAMPLES WITH MAXIMUM Cr, Zn, Mn, Cu, Mg and Fe CONCENTRATION

In summer vegetables maximum concentration of chromium in milligram per Kilogram dry weight basis was observed in the order of *Spinacea-oleracea* 116> *Momordica-charantia* 104> *Hibiscus-esculentus* local variety 70> *Hibiscus-esculentus* Indian variety 64> *Portulaca-oleracea* 24> *Luffa acutangula* 14> *Solanum-melongena* 12 (Table 2a and Figure 2a-1). While chromium content in milligram per Kilogram dry weight basis in control samples was *Spinacea-oleracea* 26, *Momordica-charantia* 14, *Hibiscus-esculentus* local variety 36, *Hibiscus-esculentus* Indian variety 40, *Portulaca-oleracea* 12, *Luffa acutangula* not detected, *Solanum-melongena* 8.

In summer vegetables maximum concentration of zinc in milligram per Kilogram dry weight basis was observed in the order of *Spinacea-oleracea* 364> *Portulaca-oleracea* 352> *Hibiscus-esculentus* Indian variety 216> *Solanum-melongena* 200> *Luffa acutangula* 188> *Momordica-charantia* 156> *Hibiscus-esculentus* local variety 152 (Table 2a & Figure 2a-2). While zinc content in milligram per Kilogram dry weight basis in control samples was *Spinacea-oleracea* 128, *Portulaca-oleracea* 290, *Hibiscus-esculentus* Indian variety 106, *Solanum-melongena* 92, *Luffa acutangula* 84, *Momordica-charantia* 104 and *Hibiscus-esculentus* local variety 116.

In summer vegetables maximum concentration of manganese in milligram per Kilogram dry weight basis was observed in the order of *Portulaca-oleracea* 184> *Hibiscus-esculentus* Indian variety 78> *Momordica-charantia* 64> *Hibiscus-esculentus* local variety 64> *Solanum-melongena* 40> *Luffa acutangula* 38 (Table 2a and Figure 2a-3). While manganese content in milligram per Kilogram dry weight basis in control samples was *Portulaca-oleracea* 158, *Hibiscus-esculentus* Indian variety 64, *Momordica-charantia* 32, *Hibiscus-esculentus* local variety 54, *Solanum-melongena* 30 and *Luffa acutangula* 28.

In summer vegetables maximum concentration of copper in milligram per Kilogram dry weight basis was observed in the order of *Momordica-charantia* 50> *Luffa acutangula*; *Solanum-melongena* and *Portulaca-oleracea* 36> *Spinacea-oleracea* 24> *Hibiscus-esculentus* Indian variety 20> *Hibiscus-esculentus* local variety 18 (Table 2a

and Figure 2a-4). While copper content in milligram per Kilogram dry weight basis in control samples was *Momordica-charantia* 10, *Luffa acutangula* 16, *Solanum-melongena* 24, *Portulaca-oleracea* 16, *Spinacea-oleracea* 16, *Hibiscus-esculentus* Indian variety 14 and *Hibiscus-esculentus* local variety 12.

In summer vegetables maximum concentration of magnesium in milligram per Kilogram dry weight basis was observed in the order of *Portulaca-oleracea* 24840> *Spinacea-oleracea* 22920> *Momordica-charantia* 4012> *Luffa acutangula* 3862> *Hibiscus-esculentus* local variety 3758> *Hibiscus-esculentus* Indian variety 3484> *Solanum-melongena* 2286 (Table 2a and Figure 2a-5). While magnesium content in milligram per Kilogram dry weight basis in control samples was *Portulaca-oleracea* 24180, *Spinacea-oleracea* 14120, *Momordica-charantia* 3836, *Luffa acutangula* 2900, *Hibiscus-esculentus* local variety 3272, *Hibiscus-esculentus* Indian variety 2876 and *Solanum-melongena* 2152.

In summer vegetables maximum concentration of iron in milligram per Kilogram dry weight basis was observed in the order *Spinacea-oleracea* 3994> *Portulaca-oleracea* 2792> *Momordica-charantia* 1396> *Hibiscus-esculentus* local variety 382> *Hibiscus-esculentus* Indian variety 376> *Solanum-melongena* 292 (Table 2a and Figure 2a-6). While iron content in milligram per Kilogram dry weight basis in control samples was *Spinacea-oleracea* 2852, *Portulaca-oleracea* 2768, *Momordica-charantia* 888, *Hibiscus-esculentus* local variety 294, *Hibiscus-esculentus* Indian variety 356 and *Solanum-melongena* 236.

Table 2 a
Summer Vegetable Samples with Maximum Cr, Zn, Mn, Cu, Mg and Fe
Concentration

a. Samples with Maximum Chromium Concentration				
S.No	Samples	Sub-box with Maximum Concentration	Supplementation Stage	Maximum Concentration (mg/Kg) Dry Weight Basis
1.	<i>Spinacea-oleracea</i> (Spinach)	(5)	1 st	116
2.	<i>Momordica-charantia</i> .(Bitter gourd)	(5)	1 st	104
3.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(1)	1 st	70
4.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(9)	2 nd	64
5.	<i>Portulaca-oleracea</i> .(Pot purslane)	(5)	1 st	24
6.	<i>Luffa acutangula</i> .(Bath sponge)	(5)	1 st	14
7.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(5,7)	1 st	12
b. Samples with Maximum Zinc Concentration				
1.	<i>Spinacea-oleracea</i> (Spinach)	(6)	1 st	364
2.	<i>Portulaca-oleracea</i> .(Pot purslane)	(6)	1 st	352
3.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(9)	1 st	216
4.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(6)	1 st	200
5.	<i>Luffa acutangula</i> .(Bath sponge)	(6)	1 st	188
6.	<i>Momordica-charantia</i> .(Bitter gourd)	(6)	1 st	156
7.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(10)	2 nd	152
c. Samples with Maximum Manganese Concentration				
1.	<i>Portulaca-oleracea</i> .(Pot purslane)	(2)	1 st	184
2.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(4)	1 st	78
3.	<i>Momordica-charantia</i> .(Bitter gourd)	(4)	1 st	64
4.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(4)	1 st	64
5.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(4)	1 st	40
6.	<i>Luffa acutangula</i> .(Bath sponge)	(2,4)	1 st	38
d. Samples with Maximum Copper Concentration				
1.	<i>Momordica-charantia</i> .(Bitter gourd)	(4)	1 st	50
2.	<i>Luffa acutangula</i> .(Bath sponge)	(4)	1 st	36
3.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(4)	1 st	36
4.	<i>Portulaca-oleracea</i> .(Pot purslane)	(1)	1 st	36
5.	<i>Spinacea-oleracea</i> (Spinach)	(4)	1 st	24
6.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(4)	1 st	20
7.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(1)	1 st	18
e. Samples with Maximum Magnesium Concentration				
1.	<i>Portulaca-oleracea</i> .(Pot purslane)	(1)	1 st	24840
2.	<i>Spinacea-oleracea</i> (Spinach)	(1)	1 st	22920
3.	<i>Momordica-charantia</i> .(Bitter gourd)	(2)	1 st	4012
4.	<i>Luffa acutangula</i> .(Bath sponge)	(1)	1 st	3862
5.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(4)	1 st	3758
6.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(2)	1 st	3484
7.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(5)	1 st	2286
f. Samples with Maximum Iron Concentration				
1.	<i>Spinacea-oleracea</i> (Spinach)	(4)	1 st	3994
2.	<i>Portulaca-oleracea</i> .(Pot purslane)	(4)	1 st	2792
3.	<i>Momordica-charantia</i> .(Bitter gourd)	(4)	1 st	1396
4.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	(4)	1 st	382
5.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	(3)	1 st	376
6.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	(4)	1 st	292

Figure 2a
Summer Vegetables with Maximum Concentration of Cr, Zn, Mn, Cu, Mg and Fe
mg/Kg Dry Weight Basis

Figure 2a-1

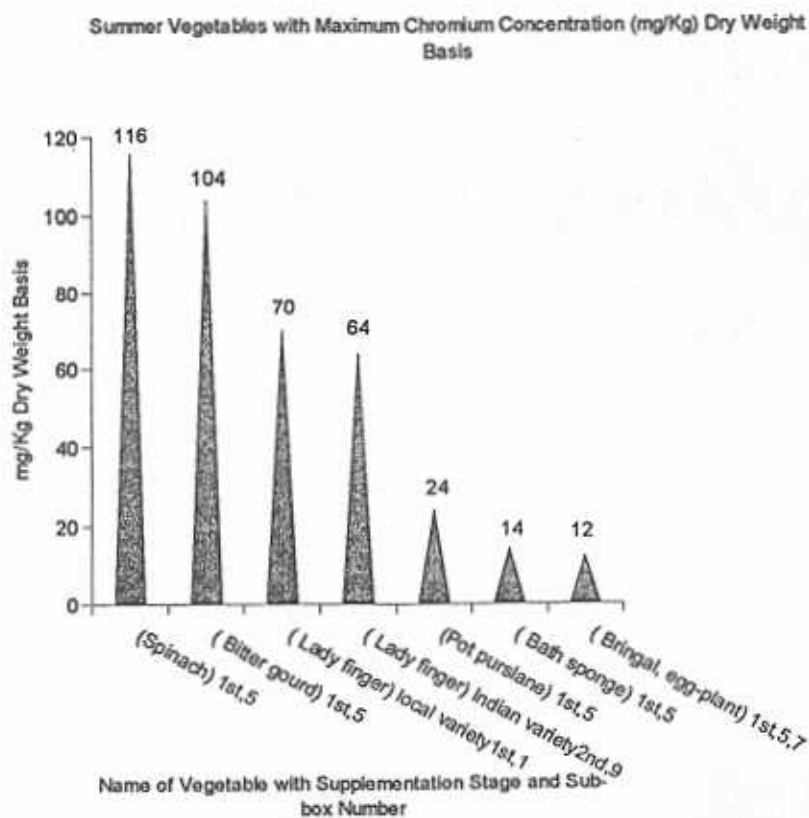


Figure 2a-2

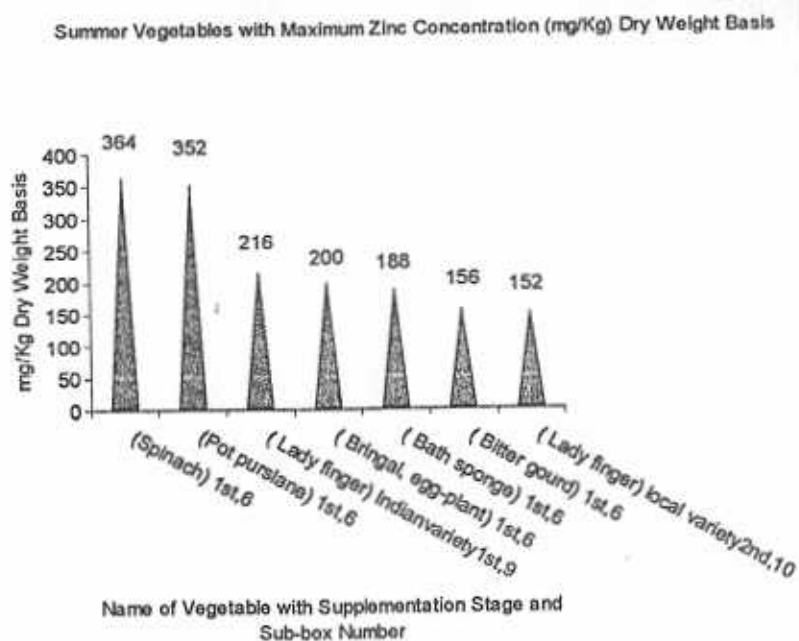


Figure 2a Continue.....

Figure 2a-3

Summer Vegetables with Maximum Manganese Concentration (mg/Kg) Dry Weight Basis

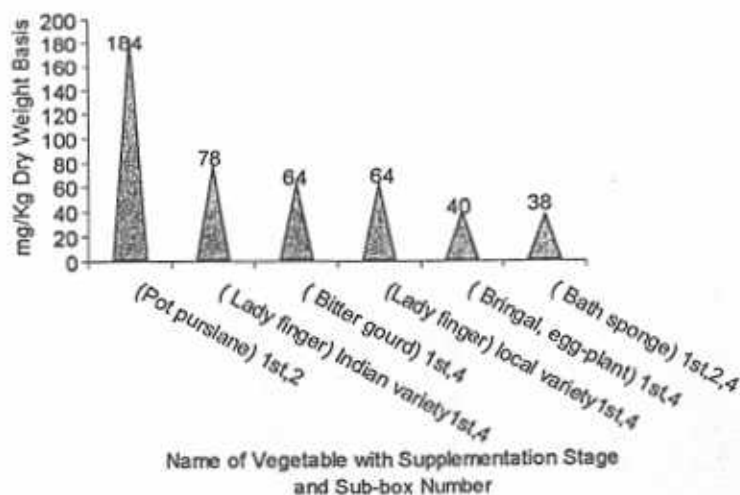


Figure 2a-4

Summer Vegetables with Maximum Copper Concentration (mg/Kg) Dry Weight Basis

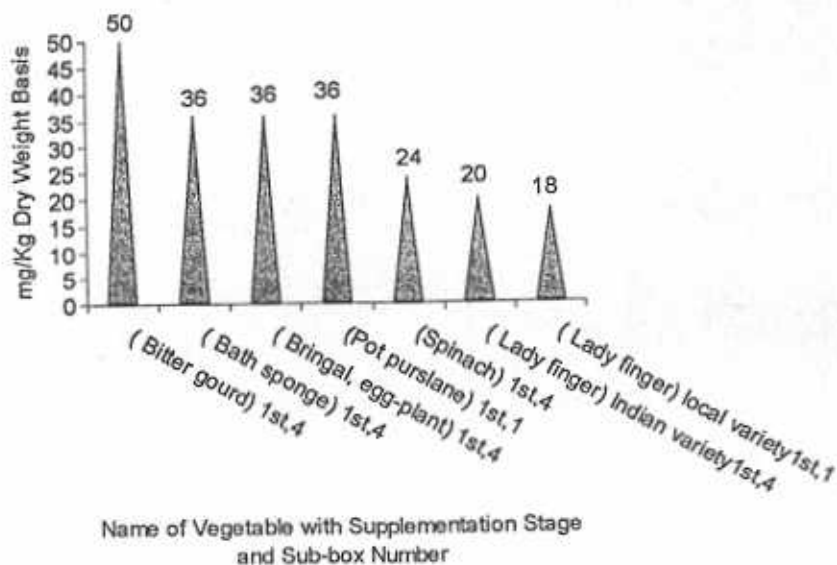


Figure 2a Continue.....

Figure 2a-5

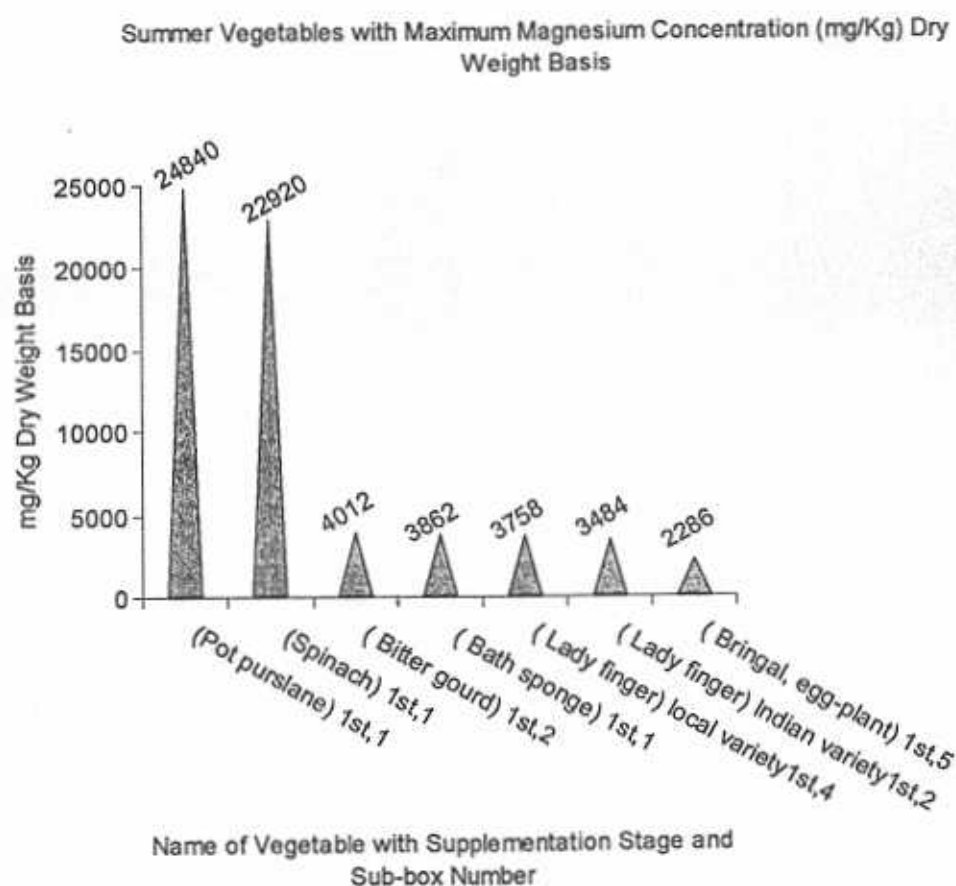
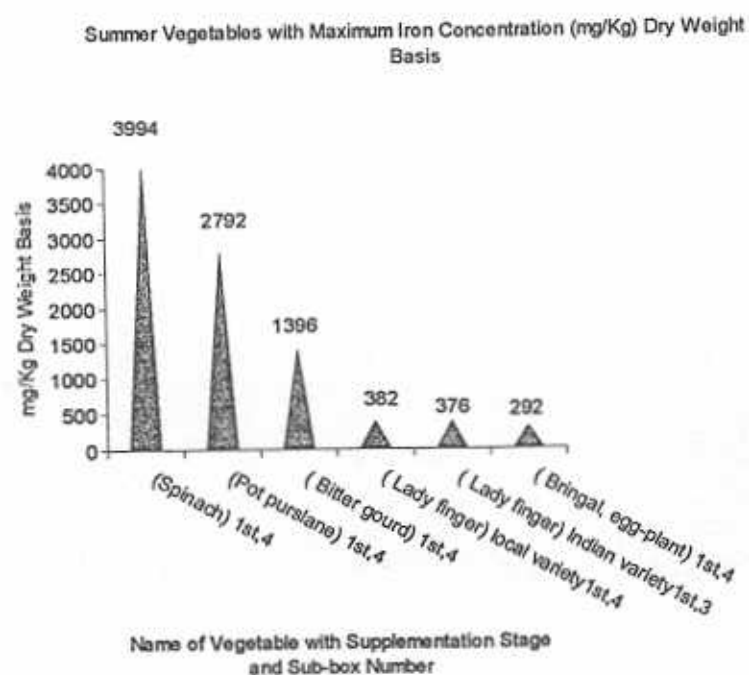


Figure 2a-6



SUMMER VEGETABLE SAMPLES WITH MAXIMUM INCREASE of Cr, Zn, Mn, Cu, Mg and Fe

Vegetables with total increase in chromium concentration milligram per Kilogram dry weight basis observed in the order *Momordica-charantia* 90> *Spinacea-oleracea* 90> *Hibiscus-esculentus* local variety 34> *Hibiscus-esculentus* Indian variety 24> *Luffa acutangula* 14> *Portulaca-oleracea* 12> *Solanum-melongena* 4 (Table 2b and Figure 2b-1).

Vegetables with total increase in zinc concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 236> *Hibiscus-esculentus* Indian variety 110> *Solanum-melongena* 108> *Luffa acutangula* 104> *Portulaca-oleracea* 62> *Momordica-charantia* 52> *Hibiscus-esculentus* local variety 36 (Table 2b and Figure 2b-2).

Vegetables with total increase in manganese concentration milligram per Kilogram dry weight basis observed in the order *Momordica-charantia* 32> *Portulaca-oleracea* 26> *Hibiscus-esculentus* Indian variety 14> *Luffa acutangula*; *Solanum-melongena* and *Hibiscus-esculentus* local variety 10 (Table 2b and Figure 2b-3).

Vegetables with total increase in copper concentration milligram per Kilogram dry weight basis observed in the order of *Momordica-charantia* 40> *Luffa acutangula* and *Portulaca-oleracea* 20> *Solanum-melongena* 12> *Spinacea-oleracea* 8> *Hibiscus-esculentus* local variety and *Hibiscus-esculentus* Indian variety 6 (Table 2b and Figure 2b-4).

Vegetables with total increase in magnesium concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 8800> *Luffa acutangula* 962> *Portulaca-oleracea* 660> *Hibiscus-esculentus* Indian variety 608> *Hibiscus-esculentus* local variety 486> *Momordica-charantia* 176> *Solanum-melongena* 134 (Table 12b and Figure 2b-5).

Vegetables with total increase in iron concentration milligram per Kilogram dry weight basis found were in the order of *Spinacea-oleracea* 1142> *Momordica-charantia* 508> *Hibiscus-esculentus* local variety 88> *Solanum-melongena* 56> *Portulaca-oleracea* 24> *Hibiscus-esculentus* Indian variety 20 (Table 2b and Figure 2b-6).

Table 2 b
Summer Vegetable Samples with Maximum Increase of Cr, Zn, Mn, Cu, Mg and Fe

a.	Samples with Maximum Chromium Increase	
S.No	Samples	(mg/Kg) Dry Weight Basis
1.	<i>Momordica-charantia</i> .(Bitter gourd)	90
2.	<i>Spinacea-oleracea</i> (Spinach)	90
3.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	34
4.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	24
5.	<i>Luffa acutangula</i> .(Bath sponge)	14
6.	<i>Portulaca-oleracea</i> .(Pot purslane)	12
7.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	4
b.	Samples with Maximum Zinc Increase	
1.	<i>Spinacea-oleracea</i> (Spinach)	236
2.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	110
3.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	108
4.	<i>Luffa acutangula</i> .(Bath sponge)	104
5.	<i>Portulaca-oleracea</i> .(Pot purslane)	62
6.	<i>Momordica-charantia</i> .(Bitter gourd)	52
7.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	236
c.	Samples with Maximum Manganese Increase	
1.	<i>Momordica-charantia</i> .(Bitter gourd)	32
2.	<i>Portulaca-oleracea</i> .(Pot purslane)	26
3.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	14
4.	<i>Luffa acutangula</i> .(Bath sponge)	10
5.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	10
6.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	10
d.	Samples with Maximum Copper Increase	
1.	<i>Momordica-charantia</i> .(Bitter gourd)	40
2.	<i>Luffa acutangula</i> .(Bath sponge)	20
3.	<i>Portulaca-oleracea</i> .(Pot purslane)	20
4.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	12
5.	<i>Spinacea-oleracea</i> (Spinach)	8
6.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	6
7.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	6
e.	Samples with Maximum Magnesium Increase	
1.	<i>Spinacea-oleracea</i> (Spinach)	8800
2.	<i>Luffa acutangula</i> . (Bath sponge)	962
3.	<i>Portulaca-oleracea</i> .(Pot purslane)	660
4.	<i>Hibiscus-esculentus</i> . (Lady finger) Indian variety	608
5.	<i>Hibiscus-esculentus</i> . (Lady finger) local variety	486
6.	<i>Momordica-charantia</i> .(Bitter gourd)	176
7.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	134
f.	Samples with Maximum Iron Increase	
1.	<i>Spinacea-oleracea</i> (Spinach)	1142
2.	<i>Momordica-charantia</i> .(Bitter gourd)	508
3.	<i>Hibiscus-esculentus</i> .(Lady finger) local variety	88
4.	<i>Solanum-melongena</i> .(Bringal, egg-plant)	56
5.	<i>Portulaca-oleracea</i> .(Pot purslane)	24
6.	<i>Hibiscus-esculentus</i> .(Lady finger) Indian variety	20

Figure 2b
Summer Vegetables with Increase in Concentration of Cr, Zn, Mn, Cu, Mg and Fe
mg/Kg Dry Weight Basis

Figure 2b-1

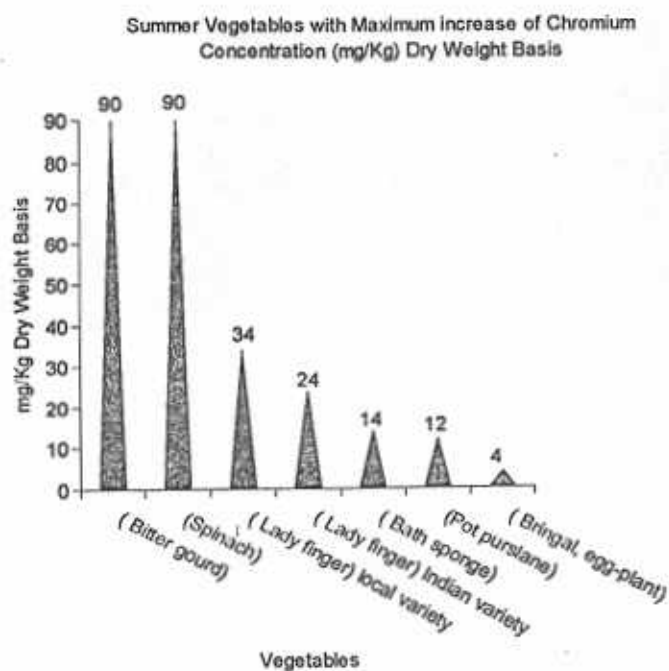


Figure 2b-2

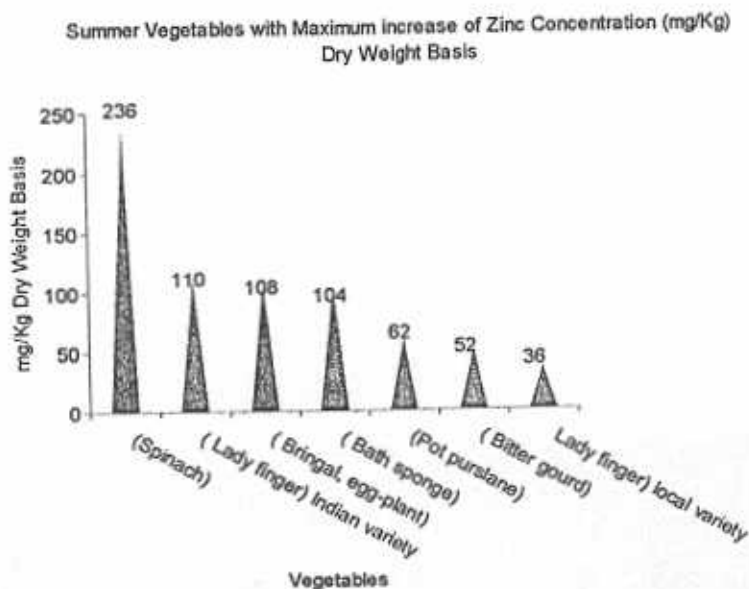


Figure 2b Continue.....

Figure 2b-3

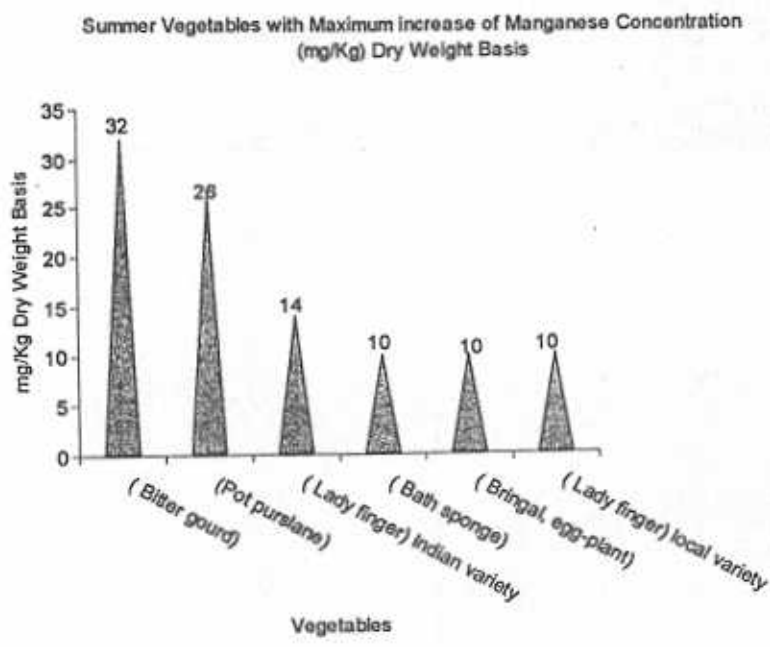


Figure 2b-4

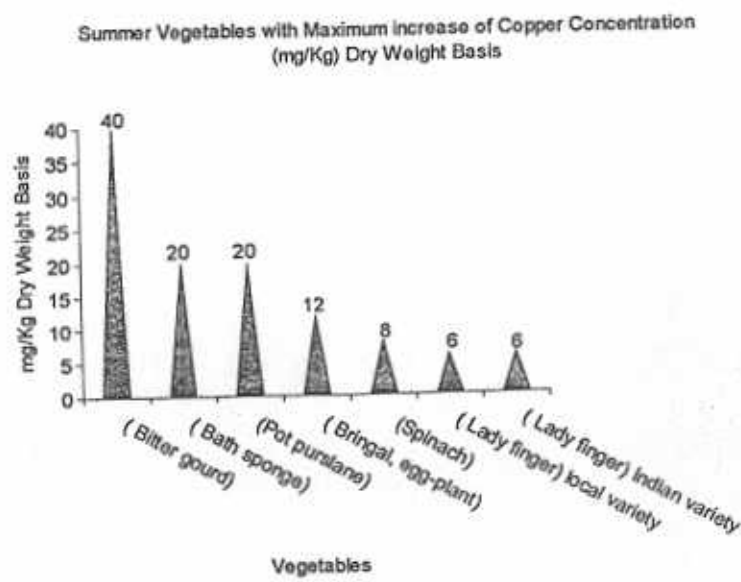


Figure 2b Continue.....

Figure 2b-5

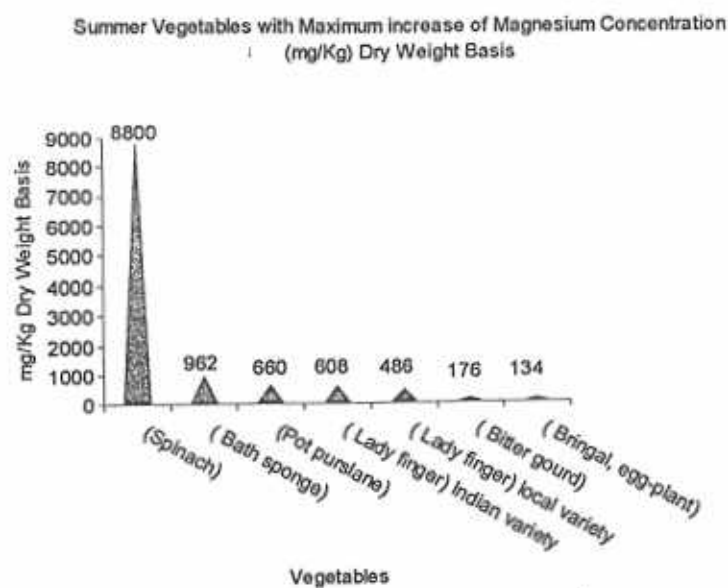
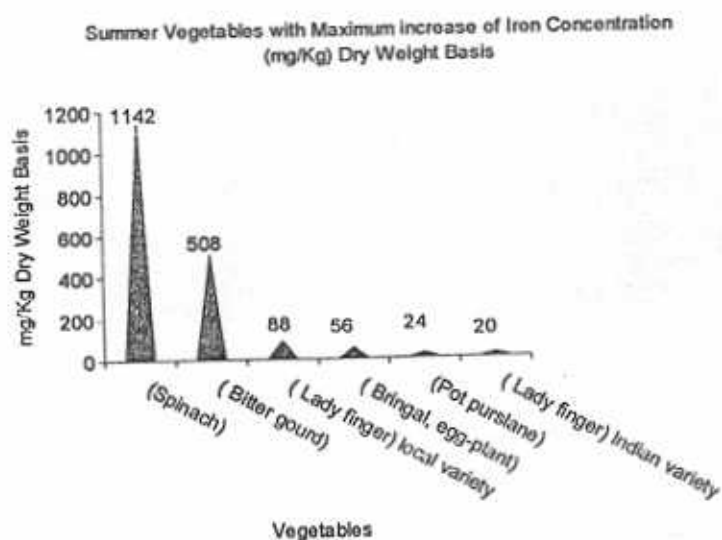


Figure 2b-6



CHAPTER-4

DISCUSSION

In the present work the main idea was enrichment of vegetables with trace metals for their role as hypoglycemic agents in the treatment of diabetes as well as other deficiency diseases. For this purpose selected vegetables from winter and summer seasons were grown on experimental basis in the soil medium. The soil type was clay loam as per soil test. Thus the soil type, other characteristics and the actual concentration of the minerals under study was same for all the vegetable samples.

Vegetative roots absorb nutrient elements from soil that are then returned to surface through leaf fall. The uptake of heavy metals by vegetable depends on the mobility and availability of metals in the soil. Factors that affect the mobility of heavy metals including: 1) chemical parameters such as pH, Eh (redox potential), organic matter content, salinity, concentrations of competing ions, ionic or anionic complexes, moisture, 2) physical parameters such as texture (size distribution) plus structure and penetrability, presence of one or an assemblage of clay minerals, their surface areas and cation exchange capacities, climate (temperature) and 3) biological parameters such as microbial activity, vegetative root extension and penetration, and burrowing organisms (Siegel, 1998).

Trace mineral concentrations and availability are affected by four interdependent factors: 1) the genus, species, or variety of crop, 2) type and mineral concentration of soil, 3) stage of plant maturity, 4) climatic or seasonal conditions (Berger, 1995).

FACTORS AFFECTED ABSORPTION OF Cr, Zn, Mn, Cu, Mg and Fe IN SELECTED VEGETABLES OF WINTER AND SUMMER SEASON

EFFECT OF CONCENTRATION

From results of chromium and zinc of both winter and summer season vegetables, it is evident that by increasing soil concentration of these elements, their concentration could be increased to a significant level. The chromium uptake by plants has been found to be positively correlated to chromium application by many workers (Kick and Braun, 1977; Zlatareva *et al.*, 1999; Tang *et al.*, 2001; Samantaray *et al.*, 2001; Zurayk *et al.*, 2001).

The best way and stage of application which can be recommended on the basis of our results for chromium and zinc supplementation is their individual application at early stage.

Plants grown in heavy metal contaminated soil often show metal accumulation, particularly in root tissue and the rate of Cr uptake is dependent on several soil and plant factors (Kabata-Pendias, 2001). The mechanism involved in the uptake and translocation of chromium in plants is not understood, because of uncertainty about the ionic species present in different systems. There is, however, evidence that Cr^{+6} is reduced to Cr^{+3} between the root surface and shoots and that irrespective of the form in which it is supplied most of the chromium is retained in roots (Wild, 1988).

The results obtained in case of zinc are in accordance with the study carried out; Zinc concentration in wheat grown with the aid of Zn-containing fertilizers was about twice that of wheat grown on the same soils with out Zn applications (Hambidge *et al.*, 1986).

The early stage is most favourable for zinc uptake because Zn declines as plants mature (Underwood, 1981).

The results obtained of both winter and summer vegetables for magnesium indicates that increasing magnesium concentration has no effect in increasing its concentration. The reason is, that magnesium being a macro element was present in appreciable amount in the soil and also supplied through water. From our results magnesium content was enhanced in the samples where chromium and zinc were applied individually or in the form of combinations. This shows that both chromium and zinc have synergistic effects on magnesium absorption by plants. Thus chromium and zinc supplementation could have good effect on the absorption of magnesium. However, the maximum magnesium absorption was recorded in samples supplemented at early stage. Magnesium concentration generally decline as the plant matures (Allcroft, 1954).

Copper, Manganese and Iron were supplemented in the form of combinations (1, 4); (2, 4); (3, 4) respectively. On the basis of our results, in case of copper combination (4) is best for summer and combination (1) for winter vegetables. While in case of manganese combination (4) is best for the vegetables of both seasons. Similarly, in case of iron combination (4) is best for summer vegetables. As iron was also present in quite good and high concentration, therefore, its uptake in winter season vegetables was different. Maximum iron concentration was recorded in the presence of chromium supplementation. Thus chromium has synergistic effects in the uptake of iron.

The application of Cu-containing fertilizers to soils low in plant-available-Cu invariably increases the Cu concentration in the herbage and often increases yield (Underwood, 1981).

Overall in winter and summer vegetables, where copper and zinc was supplemented, there manganese and iron concentration was decreased. This indicates that high soil concentrations of copper or zinc or both can also induce iron and manganese deficiency symptoms. Copper and zinc are mutually competitive as well as competitive to other micronutrients at the carrier sites for plant root uptake (Tisdale, 1985).

In case of winter vegetables the application of combination (4) containing Cu and Zn decreased concentration of other metals under study, except chromium, while their effect in summer vegetables was different that is no decrease of other metals was occurred.

EFFECT OF SEASON

For comparison purpose two vegetables, *Spinacea-oleracea* and *Portulaca-oleracea* were grown both in winter and summer seasons. In case of *Spinacea-oleracea* comparing control results, chromium and manganese content was high in summer while Zn, Cu, Mg and Fe content was high in winter. Chromium, zinc, copper, magnesium and iron contents were increased in all summer vegetables. The increase is quite significant suggesting that summer season is more suitable for enhancing the contents of these minerals than winter. Similarly, the manganese content of control samples of summer is too high, that no further increase occurred after supplementation.

Climate may influence plant concentration as prevalence of Zn deficiency in cool, wet seasons has been associated with decreased solubility of soil Zn (Reuter, 1975).

Metals accumulation in plant depends on plant species, growth stages, type of soil, metals, soil conditions, weather and environment (Chang, 1984 and Petruzzelli, 1989).

In case of *Portulaca-oleracea* comparing control results, chromium and iron content was higher in winter vegetables, while Zn, Mn, Cu and Mg content was higher in summer vegetables. Enhancement of chromium and iron content is also greater in winter than summer season. Another important finding is significant increase in zinc, magnesium and iron contents after supplementation. Zinc, Manganese, copper, and magnesium content was increased in all summer vegetables. The results suggest that for enhancing chromium, magnesium and iron contents, winter season is best. Similarly, for Zinc, Manganese, copper, and magnesium summer season is best.

EFFECT OF VARIETY

Different crop species have different micronutrient requirements just as they do for other elements and even varieties within a species differ in their ability to absorb and translocate the various nutrients (Shuman, 1999). Plants within the same species vary in their ability to absorb nutrients (Munson, 1969).

In our study two varieties of *Hibiscus-esculentus* local and Indian were grown in the summer season to observe the minerals absorption capability. The results obtained show that same plant with different varieties has different tendency and capability to assimilate the minerals. The behavior towards supplemented minerals was different, such as increase in chromium concentration was nearly same, but in case of local variety increase occurred where chromium supplemented in the form of combination (1) and in case of Indian variety increase occurred where chromium supplemented individually (9). The same tendency was observed for all selected elements except manganese.

EFFECT OF PLANT PARTS

In case of *Raphanus-sativus* and *Brassica-rapa* much variation in mineral content between roots and leaves was observed. In *Raphanus-sativus* concentration of Cr, Zn, Cu, and Fe was remained low than leaves of control sample, but after supplementation both root and leaves concentration was enhanced almost to the same level. The Mg concentration in root and leaves of control and supplemented samples remained. Because magnesium is part of chlorophyll, the green pigment in plants, green leafy vegetables are rich in magnesium (Food and Nutrition Board, 2001).

Similarly, in *Brassica-rapa* concentration of Cr, Zn, Mn, Cu, Mg and Fe was higher in leaves than root in control sample. After supplementation with these minerals, maximum chromium content observed was the same in roots and leaves, while Zn, Cu and Fe maximum contents observed are higher in leaves. Therefore it is suggested that leaves which many people do not utilize, should not be discarded being good source of minerals.

THE CONCENTRATION OF Cr, Zn, Mn, Cu, Mg AND Fe IN EACH VEGETABLE UNDER STUDY AND THEIR THERAPEUTIC AND NUTRITIONAL ROLE ON THE BASIS OF RECOMMENDED DAILY ALLOWANCES

The recommended daily allowances established for Cr, Zn, Mn, Cu, Mg and Fe have been given in (Table 1.1); (Table 1.2) ; (Table 1.4); (Table 1.6) ; (Table 1.8) ; and (Table 1.10) respectively. While tolerable upper intake level of Zn, Mn, Cu, Mg and Fe have been given in (Table 1.3); (Table 1.5); (Table 1.7), (Table 1.9) and (Table 1.11) respectively (Chapter-1).

The vegetables under study have been graded on the basis of maximum and total increase in the concentration of Cr, Zn, Mn, Cu, Mg and Fe. In case of each vegetable, comparison has been made with respect to control sample indicating difference and extent of contribution in recommended daily allowance (FOOD AND NUTRITION BOARD 2001) of these elements with and without supplementation. Also vegetables have been sorted as good contributing source in the treatment of diabetes on the basis of higher contents of Cr, Zn, Mg and copper, which are reported hypoglycemic agents and can be provided in lesser serving size or concentrated form to fulfill the required amount helpful in the management of diabetes. Assuming that single serving of 100gram fresh vegetable will have an average of 90 % moisture and 10 gram dry material.

CHROMIUM IN WINTER AND SUMMER VEGETABLES

The average dietary Cr intake seems to fluctuate considerably among countries. In many developing countries dietary intake is high, from 50-100 micrograms per day, whereas in developed countries, such as Finland, Sweden, Switzerland, and the US, average intake is 50 micrograms per day or lower and consequently at or below the estimated safe and adequate daily dietary intake (ESADDI) range of 50-200 micrograms per day established by the National Academy of Sciences (Kumpulainen, 1992).

The chromium recommended daily allowances in case of males and females ranges; infants (0.2-5.5mcg/day), children (11-25mcg/day), adolescent (Male:

35mcg/day, Female: 24mcg/day), adults (Male: 30 - 35 mcg/day, Female: 20-25mcg/day), pregnancy (29-30 mcg/day) and increasing demand in stage of breastfeeding (44-45 mcg/day) (Table 1.1).

The extra contribution of chromium by winter vegetables after supplementation

In winter vegetables total increase in chromium concentration milligram per Kilogram dry weight basis has been shown in the order (*Portulaca-oleracea* 40> *Spinacea-oleracea* 20> *Brassica-rapa* Roots 18>*Brassica-rapa* Leaves16 > *Allium-sativum* 14 > *Brassica-campestris-var-sarson* 12> *Trigonella-foenum-graceum*10 > *Raphanus-sativus* Leaves and Roots 6) (Table 1b and Figure 1b-1).

From the results even obtained in case of control samples the Cr concentration is too good that one serving of 100 gram fresh sample can fulfill the daily requirement of adult male/female. Because the soil used for experimental purpose was never used for agricultural purpose and had good concentration of this element.

In supplemented samples, the sample with lowest concentration of chromium is *Raphanus-sativus* Roots 14 milligram per Kilogram or 14000 microgram per Kilogram dry weight basis (Table 1a) while in control samples only two samples are above this concentration. Thus only three samples can have good contribution in diabetes on the basis of chromium content in control samples. From the supplemented vegetable result it is inferred that *Portulaca-oleracea*, *Spinacea-oleracea*, *Trigonella-foenum-graceum*, *Brassica-rapa* Roots and Leaves , *Allium-sativum* , *Brassica-campestris-var-sarson* are the best source of chromium and can provide the requisite minimum amount of 200mcg of chromium in the management of diabetes by taking small serving size of these vegetables, though *Raphanus-sativus* Leaves and Roots are also good source of chromium.

Among winter vegetables, leafy vegetables *Portulaca-oleracea* and *Spinacea-oleracea* (Figure 1a-1) showed their great affinity for chromium uptake and could be considered as a good contributing source in the diabetes management. Vegetables, especially leafy vegetables, accumulate higher amounts of heavy metals because of the fact that they absorb these metals in their leaves (Sharma and Kansal, 1986).

The extra contribution of chromium by summer vegetables after supplementation

In summer vegetables total increase in chromium concentration milligram per Kilogram dry weight basis observed in the order *Momordica-charantia* 90> *Spinacea-oleracea* 90> *Hibiscus-esculentus* local variety34> *Hibiscus-esculentus* Indian variety24> *Luffa acutangula* 14> *Portulaca-oleracea* 12> *Solanum-melongena* 4 (Table 2b and Figure 2b-1).

The chromium content observed in control samples of summer vegetables is also quite good, except *Solanum-melongena* 8 mg/Kg dry weight basis and *Luffa acutangula* in which no chromium detected. In summer vegetables *Spinacea-oleracea*, *Momordica-charantia*, *Hibiscus-esculentus* local variety, *Hibiscus-esculentus* Indian variety, *Portulaca-oleracea* can be considered as the best source, while *Luffa acutangula* and *Solanum-melongena* as good source of chromium. Moreover, *Spinacea-oleracea* and *Momordica-charantia* with such high chromium content can play more important role in the treatment of diabetes by taking very small amount of these vegetables.

Chromium (Cr) deficiency is a causative factor for NIDDM, producing symptoms including fasting hyperglycemia, impaired glucose tolerance, decreased insulin binding and receptor number, decreased HDL, and increased total cholesterol and triglycerides (Anderson, 1995).

Insufficient dietary intake of Cr leads to signs and symptoms that are similar to those observed for diabetes and cardiovascular diseases. Many chromium dietary supplements are currently available to alleviate this deficiency (Stearns, 1995).

The rural and urban populations in Iraq use bread made from barley flour for the management of diabetes mellitus (Mahdi, 1991). This observation led Ghanim and Naismith to investigate the effects of barley on diabetic rats. They found that rats fed with barley had considerable lower glucose concentration than those, which did not receive barley. Analysis of the various constituents of barley revealed its high content of chromium containing 10 times as much brewer s yeast, formerly believed to be the

richest natural source of chromium picolinate (Schroedder, 1962). Ghanin and Naismith concluded that the beneficial properties of Iraqi barley must reside in its high content of chromium. Experimental chromium deficiency in rats leads to diminished glucose tolerance and eventually to a state resembling diabetes mellitus (Schwartz, 1959).

Chromium appears to enhance the action of insulin and chromium deficiency has resulted in impaired glucose tolerance. Chromium insufficiency has been hypothesized to be a contributing factor to the development of Type-2 diabetes (Food and Nutrition Board 2001 and Jeejeebhoy, 1999).

ZINC IN WINTER AND SUMMER VEGETABLES

The zinc recommended daily allowances in case of males and females ranges; infants (2-3mg/day), children (3-8mg/day), adolescent (Male: 11mg/day, Female: 9mg/day), adults (Male: 11 mg/day, Female: 8mg/day), pregnancy (11-12 mg/day) and breastfeeding (12-13 mg/day) (Table 1.2).

Tolerable upper intake level (UL) for zinc is; infants (4-5mg/day), children (7-23mg/day), adolescent (34mg/day), adults (40mg/day) (Table 1.3).

The extra contribution of Zinc by winter vegetables after supplementation

In winter vegetables total increase in zinc concentration milligram per Kilogram dry weight basis observed in the order of *Portulaca-oleracea* 228> *Trigonella-foenum-graceum* 94> *Brassica-rapa* Roots 68> *Spinacea-oleracea* 66> *Brassica-rapa* Leaves 64> *Raphanus-sativus* Roots 62> *Raphanus-sativus* Leaves 44> *Brassica-campestris-var-sarson* 30> *Allium-sativum* 28. (Table 1b and Figure 1b-2)

The extra contribution of Zinc by summer vegetables after supplementation:

In summer vegetables total increase in zinc concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 236> *Hibiscus-esculentus* Indian variety 110> *Solanum-melongena* 108> *Luffa acutangula*

104> *Portulaca-oleracea* 62> *Momordica-charantia* 52> *Hibiscus-esculentus* local variety 36 (Table 2b and Figure 2b-2).

From the above results of winter and summer vegetables showing significant increase of zinc after supplementation, it is obvious that these have the capability to accumulate zinc in higher concentration provided the soil zinc content is high. The maximum daily recommended requirement of zinc in case of adult male and female is 11 and 8 milligram per day respectively which can be easily fulfilled by taking lesser amount of these vegetables after supplementation. The increase is not too much high to pose any health hazard problems in normal individuals and would also be beneficial for diabetic individuals to get reasonable amount of zinc daily by consuming lesser amount of these vegetables.

Zinc plays a role in the synthesis of insulin by pancreatic beta cells (Emdin, *et al.* 1980) and in the action of insulin at the cellular level (Arquilla, *et al.* 1978 and Herington, 1985).

Diabetics often have impaired immune function (manifesting as increased susceptibility to infection), which may be due in part to zinc deficiency. Administration of zinc to diabetic patients increases the T lymphocyte response to phyto hem agglutinin (a measure of immune-system function) (Niewoehner CB, *et al.* 1986). Zinc also plays a crucial role in wound healing, which is abnormal in many diabetics.

Zinc supplementation reduces blood sugar in diabetics (Gupta R, 1998; Anderson RA, *et al.* 2001; Kojima, *et al.* 2002; Song, *et al.* 2003; Roussel *et al.* 2003).

MANGANESE IN WINTER AND SUMMER VEGETABLES

The manganese recommended daily allowances in case of males and females ranges; infants (0.003-0.6mg/day), children (Male: 1.2-1.9mg/day, Female: 1.2-1.6mg/day), adolescent (Male: 2.2mg/day, Female: 1.6mg/day), adults (Male:

2.3mg/day, Female: 1.8mg/day), pregnancy all ages (2.0mg/day) and also breastfeeding all ages (2.6 mg/day) (Table 1.4).

Tolerable upper intake level (UL) for manganese is; infants (Not possible to establish), children (2-6mg/day), adolescent (9mg/day) and adults (11mg/day) (Table 1.5).

The extra contribution of Manganese by winter vegetables after supplementation

In winter vegetables total increase in manganese concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 32> *Brassica-campestris-var-sarson* 22> *Portulaca-olerace* 20> *Trigonella-foenum-graceum* 10> *Allium-sativum* 4 (Table 1b and Figure 1b-3).

The extra contribution of Manganese by summer vegetables after supplementation

In summer vegetables total increase in manganese concentration milligram per Kilogram dry weight basis observed in the order *Momordica-charantia* 32> *Portulaca-oleracea* 26> *Hibiscus-esculentus* Indian variety 14> *Luffa acutangula*; *Solanum-melongena* and *Hibiscus-esculentus* local variety 10 (Table 2b and Figure 2b-3).

The results obtained in case of both winter and summer vegetables are in safe limit. Manganese toxicity resulting from foods alone has not been reported in humans, even though certain vegetarian diets could provide up to 20 mg/day of manganese (Food and Nutrition Board 2001 and Keen, 1994).

Neither 15 mg nor 30 mg of oral manganese improved glucose tolerance in diabetics or non-diabetic controls when given at the same time as an oral glucose challenge (Walter, Aoki, 1991). Although manganese appears to play a role in glucose metabolism, there is little evidence that manganese supplementation improves glucose tolerance in diabetic or non-diabetic individuals.

COPPER IN WINTER AND SUMMER VEGETABLES

The copper recommended daily allowances in case of males and females ranges; infants (200-220mcg/day), children (340-700mcg/day), adolescent (890mcg/day), adults (900mcg/day), pregnancy all ages (1000mcg/day) and also breastfeeding all ages (1300 mcg/day) (Table 1.6).

Tolerable upper intake level (UL) for manganese is; infants (Not possible to establish), children (1,000-5000mcg/day), adolescent (8,000mcg/day) and adults (10,000mcg/day) (Table 1.7).

The extra contribution of Copper by winter vegetables after supplementation

In winter vegetables total increase in copper concentration milligram per Kilogram dry weight basis was in the order of *Raphanus-sativus* Leaves 32> *Raphanus-sativus* Roots 24> *Portulaca-oleracea* 8> *Brassica-rapa* Leaves 6> *Brassica-rapa* Roots and *Allium-sativum* 4 (Table 1b and Figure 1b-4).

The extra contribution of Copper by summer vegetables after supplementation

In summer vegetables total increase in copper concentration milligram per Kilogram dry weight basis observed in the order of *Momordica-charantia* 40> *Luffa acutangula* and *Portulaca-oleracea* 20> *Solanum-melongena* 12> *Spinacea-oleracea* 8> *Hibiscus-esculentus* local variety and *Hibiscus-esculentus* Indian variety 6 (Table 2b and Figure 2b-4).

Among winter vegetables only *Raphanus-sativus* (leaves and roots), while in summer vegetables *Momordica-charantia*, *Luffa acutangula*, *Portulaca-oleracea* and *Solanum-melongena* showed significant increase in copper content. All the results are in safe limit. By consuming 200 or 250 grams of the said vegetables in fresh form can easily fulfill daily requirements of different age groups of human beings.

Two male volunteers who consumed a controlled intake of 0.7-0.8 mg of copper per day for 5-6 months had increased (Manohar, *et al*, 2002) glucose levels

during a glucose tolerance test. These levels returned toward normal after adequate amounts of copper were restored to the diet (Klevay, *et al*, 1983).

In two other volunteers, administration of 6 mg/day of copper improved glucose tolerance suggesting that their usual diet was deficient in copper (Klevay, *et al*, 1986).

A study in patients with type 2 diabetes found that those with progressive kidney disease excreted higher levels of copper in urine and had lower levels in plasma than patients with less serious disease (Ito, *et al*, 2001).

MAGNESIUM IN WINTER AND SUMMER VEGETABLES

The magnesium recommended daily allowances in case of males and females ranges; infants (30-75mg/day), children (Male: 80-240mg/day), adolescent (Male: 410mg/day, Female: 360mg/day), adults (Male: 420mg/day, Female: 320mg/day), pregnancy (350-400mg/day) and also breastfeeding (310-360 mg/day) (Table 1.8).

Tolerable upper intake level (UL) for magnesium is; infants (Not possible to establish), children (65-350mg/day), adolescent (350mg/day), adults (350mg/day) (Table 1.9).

The extra contribution of Magnesium by winter vegetables after supplementation

In winter vegetables total increase in magnesium concentration milligram per Kilogram dry weight basis observed was in the order of *Portulaca-oleracea* 9460> *Spinacea-oleracea* 1720> *Brassica-campestris-var-sarson* 424> *Trigonella-foenum-graceum* 256> *Raphanus-sativus* Roots 212> *Allium-sativum* 116 (Table 1b and Figure 1b-5).

The extra contribution of Magnesium by summer vegetables after supplementation

In summer vegetables total increase in magnesium concentration milligram per Kilogram dry weight basis observed in the order of *Spinacea-oleracea* 8800> *Luffa acutangula* 962> *Portulaca-oleracea* 660> *Hibiscus-esculentus* Indian variety 608> *Hibiscus-esculentus* local variety 486> *Momordica-charantia* 176> *Solanum-melongena* 134 (Table 12b and Figure 2b-5).

Magnesium is a macro element and required in larger amount. The results obtained are not enough good to provide maximum amount of magnesium except *Portulaca-oleracea* and *Spinacea-oleracea* with relatively high concentration. Thus the said two plants can play important role in diabetes management and also in other diseases.

Dietary magnesium supplements (400 mg/day) were found to improve glucose tolerance in elderly individuals (Paolisso, 1993). Presently, there is little scientific evidence supporting routine magnesium supplementation for diabetics. However, a few studies suggest that correcting magnesium depletion in elderly and diabetic individuals may improve glucose tolerance.

In a cross-sectional study of more than 6,000 non-diabetic adults in the U.K., those with higher fruit and vegetable intakes had significantly lower levels of glycosylated hemoglobin (HbA_{1c}), a measure of long-term blood glucose control (Sargeant, 2001). Although the relationships between fruit and vegetable intake and diabetes remain to be clarified, possible compounds in fruits and vegetables that may enhance glucose control include fiber and magnesium (Meyer, 2000).

Induction of a hypomagnesemic state in rats is known to significantly decrease sensitivity to insulin via an alteration at the insulin receptor-associated tyrosine kinase. Supplementation with magnesium has been shown to delay the development of type-2 DM in experimental animal models. Early studies demonstrated an inverse relationship between plasma magnesium levels and fasting blood glucose levels in insulin-treated patients with diabetes (McNair, 1982).

A recent human, placebo-controlled study by (DeValk, 1998) reported on the effects of supplemental magnesium (15 mmol/day for 3 months) in 50 patients with type-2 DM who required insulin. While plasma magnesium and urinary magnesium excretion increased with magnesium therapy, other parameters measured (HbA1c, blood glucose, lipids) failed to change.

However, there was a slight reduction in diastolic blood pressure in those patients experiencing increases in plasma magnesium. Although a reduced release of insulin has been reported in individuals with compromised magnesium status, most of the focus on magnesium supplementation in DM now involves interest in preventing long-term complications. Magnesium deficiency has been associated with hypertension, dyslipidemia and retinopathy, (Seelig, 1989) all common to DM.

Serum magnesium concentrations were significantly lower in a group of 56 diabetics than in healthy controls (Ceriello, *et al*, 1982). Hypomagnesemia was more pronounced in individuals with diabetic retinopathy (McNair P, *et al*, 1978) or cardiac complications (Wen-gang, *et al*, 1987) than in diabetics without such complications. Poor control of diabetes was often associated with low serum magnesium (Vanroelen, *et al*, 1985). HbA1c levels correlated significantly with Mg concentrations in plasma, muscle, and mononuclear cells (Sjogren, *et al*, 1986).

Magnesium deficiency has also been found to be common in children with type-1 diabetes (Fort, *et al*, 1986). The reduced concentration of magnesium seen in diabetics appears to result in part from increased urinary magnesium excretions (Errazti, *et al*, 1982). In addition, the American diet tends to be low in magnesium. Dietary surveys have shown that 80-85% of American women consume less than the Recommended Dietary Allowance (RDA) for this mineral (Morgan, *et al*, 1985). Daily magnesium intake in two other studies was only about two-thirds of the RDA (Lakshmanan, *et al*, 1984 and Srivastava, *et al*, 1978).

In a double-blind study of 12 non diabetic elderly individuals, magnesium supplementation was found to improve the secretion and action of insulin (Paolisso, *et*

al, 1992). In addition, a considerable body of evidence indicates that magnesium can prevent cardiovascular disease, for which diabetics are at increased risk (Gaby, 1994).

Magnesium supplementation has been found to improve insulin sensitivity and insulin secretion in patients with Type-2 diabetes (De Valk, 1999). Magnesium deficiency has been associated with insulin resistance and over all poor diabetic control in elderly individuals who are borderline diabetic (Tosiello, 1996). A newer study has found that a group of persons with type 1 diabetes had insufficient levels of magnesium dietary intake (Djurhuus, *et al*, 2001). This study also found that magnesium supplementation in these same patients led to reduced insulin-stimulated glucose uptake. Low serum levels of magnesium have also been found to be associated with complications of type-2 diabetes, such as the development of foot ulcers (Rodriguez, 2001).

Significant evidence has been found that magnesium intake is significantly associated with a reduced risk of developing diabetes (Lopez, *et al*, 2004). A new study of over weight women found that magnesium supplementation significantly reduced the risk of developing diabetes (Song Y, *et al*, 2004). In a recent study of patients with diabetes and those without the disease found that the diabetic patients had lower levels of magnesium in the blood and that magnesium supplementation stabilized blood sugar levels in those with disease (Lal, *et al*, 2003). Thus, magnesium supplementation may be an important component of the overall treatment of many diabetics.

IRON IN WINTER AND SUMMER VEGETABLES

The iron recommended daily allowances in case of males and females ranges; infants (0.27-11mg/day), children (7-10mg/day), adolescent (Male: 11mg/day, Female: 15mg/day), adults (Male: 8mg/day, Female: 18mg/day), pregnancy all ages (27mg/day) and breastfeeding (9-10 mg/day) (Table 1.10).

Tolerable upper intake level (UL) for iron is; infants (Not possible to establish), children (40mg/day), adolescent (45mg/day) and adults (45mg/day) (Table 1.11).

The extra contribution of Iron by winter vegetables after supplementation

In winter vegetables total increase in iron concentration milligram per Kilogram dry weight basis observed was in the order of *Portulaca-oleracea* 684 > *Trigonella-foenum-graceum* 520 > *Spinacea-oleracea* 374 > *Brassica-rapa* Roots 188 > *Brassica-rapa* Leaves 72 > *Allium-sativum* 8 (Table 1b and Figure 1b-6).

The extra contribution of Iron by summer vegetables after supplementation

In summer vegetables total increase in iron concentration milligram per Kilogram dry weight basis found was in the order of *Spinacea-oleracea* 1142 > *Momordica-charantia* 508 > *Hibiscus-esculentus* local variety 88 > *Solanum-melongena* 56 > *Portulaca-oleracea* 24 > *Hibiscus-esculentus* Indian variety 20 (Table 2b and Figure 2b-6).

Among winter and summer vegetables *Spinacea-oleracea* and *Portulaca-oleracea* are the best sources of iron. Thus increased iron content can compensate for the less bioavailability of this element from these plants. Significant increase was also occurred in other vegetables except *Allium-sativum*.

Most of the symptoms of iron deficiency are a result of the associated anemia, and may include fatigue, rapid heart rate, palpitations, and rapid breathing on exertion. Iron deficiency impairs athletic performance and physical work capacity in several ways. In iron deficiency anemia, the reduced hemoglobin content of red blood cells results in decreased oxygen delivery to active tissues. Decreased myoglobin levels in muscle cells limit the amount of oxygen that can be delivered to mitochondria for oxidative metabolism. Iron depletion also decreases the oxidative capacity of muscle by diminishing the mitochondrial content of cytochromes and other iron-dependent enzymes required for electron transport and ATP synthesis. Lactic acid production is also increased in iron deficiency (Beard, 2001).

The ability to maintain a normal body temperature on exposure to cold is also impaired in iron-deficient individuals. Severe iron deficiency anemia may result in brittle and spoon-shaped nails, sores at the corners of the mouth, taste bud atrophy, and a sore tongue. In some cases, advanced iron-deficiency anemia may cause difficulty in swallowing due to the formation of webs of tissue in the throat and esophagus. The development of esophageal webs, also known as Plummer-Vinson syndrome, may require a genetic predisposition in addition to iron deficiency. Pica, a behavioral disturbance characterized by the consumption of non-food items, may be a symptom and a cause of iron deficiency (Lee, 1999).

From the present study it can be concluded that by changing the soil minerals environment the uptake of required mineral content of vegetables, perhaps could be enhanced. The maximum minerals absorption and increase was manifested by vegetables at first supplementation stage. Summer season remained more favorable on the basis of percent increase of minerals than winter season. Soil type, seasonal effects and plant variety have important effect on plant minerals absorption. Thus it is possible that with some other soil type and seasonal conditions the better results could be achieved. The present study has been especially correlated with diabetes management/control on the basis of provision of enhanced level of minerals through vegetables, which can play important role in diabetes and also in other deficiency diseases like anemia etc. As almost all vegetables are easily available, thus enhancing their mineral content by supplementation would be a good source for eliminating deficiency diseases.

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