Pak. J. Biochem. Mol. Biol. 2010; 43(2): 61-71

Medicinal significance of vegetables cultivated over minerals supplemented soil

Javed Abbas Bangash[1], Abdus Sattar Khan[2], Muhammad Arif[1], Faizullah Khan[1] and Faridullah Khan[1]*
[1]PCSIR Laboratories Complex, Jamrud Road Peshawar, Pakistan
[2]Department of Chemistry, Kohat University of Science & Technology (KUST), Banu Road, Kohat, Pakistan

Abstract: Three winter season vegetables Fenugreek/Methi (*Trigonella-foenum-graceum*), Sarson (*Brassica-campestris-var-sarson*) and Garlic (*Allium-sativum*) 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. Thus calculated amount of Cr, Zn, Mn, Cu, Mg and Fe salts (as fertilizer) were applied in solution form to the roots of vegetables in different concentration as individual or in combinations. These vegetables were grown from seeds in the soil plot. After harvesting vegetables were dried, acid digested and analyzed for Cr, Mn, Zn, Cu, Fe and Mg on Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer. Thus in Fenugreek/Methi (*Trigonella-foenum-graceum*) total increase of Cr, Zn, Mn, Mg and Fe recorded was (10, 94, 10, 256 and 520) mg/Kg dry weight basis; in Sarson (*Brassica-campestris-var-sarson*) total increase of Cr, Zn, Mn and Mg recorded was (12, 30, 22 and 424) mg/Kg dry weight basis and (Garlic) (*Allium-sativum*) total increase of Cr, Zn, Mn, Cu, Mg and Fe recorded was (14, 28, 4, 4, 116 and 10) mg/Kg dry weight basis. 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. This could play important role in management of diabetes control and also in the elimination of other deficiency diseases like anemia.

Keywords: Winter vegetables, enrichment, selected minerals, enhance uptake, role in diseases.
Received: January 10, 2010 **Accepted:** March 25, 2010
***Author for Correspondence:** jbpunjee@yahoo.com

INTRODUCTION

The study of how plants absorb and assimilate inorganic ions is called mineral nutrition. In fact, yields of most crop plants increase linearly with the amount of fertilizer that they absorb[1].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[2].

The list of minerals includes the 14 mineral elements defined as essential for plant growth and reproductive success[3].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[4-5].Of these 14 elements, human essentiality has been confirmed for all but B and Ni, although circumstantial evidence for their essentiality has been reported[6]. Na, Cr, I and Se also are required by humans, but not by plants. The plants can acquire these other elements through non-specific influx processes using existing transporters localized to their roots[7]. 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[6] and thus their incorporation into plant tissues is of dietary relevance.

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[8].

The use of forage crops enriched in Se from Se enriched soils to supplement the diets of animals has been proposed[9]. 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[10].We get 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[11-12].

Another study has found that dietary supplementation with chromium can moderate glucose intolerance and control blood sugar in diabetic patients[13].

MATERIALS AND METHODS

Preparation of soil

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 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. Moreover, the sub-boxes from 1 to 12 represent one sample grown in these boxes (twelve samples of one vegetable) which was supplemented either with individual element or combination of elements except box-12. 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. 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.

Purchasing and planting seeds

Fenugreek/Methi *(Trigonella-foenum-graceum)* and Sarson *(Brassica-campestris-var-sarson)* seeds were planted about 1/2-inch deep in the soil. Two dozen seeds of each, fenugreek and sarson were sown 1-inch apart in three rows with eight seeds per row. For planting Garlic (*Allium-sativum*), 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.

Application of fertilizer

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

Application pattern of Fe, Zn, Mn, Cr, Mg, and Cu to soil

Solution of each Fe, Zn, Mn, Cr, Mg, and Cu salts 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
Sub-boxes:
A-1……………. Com-1. Cr, Cu, Mg
A-2……………. Com-2. Cr, Mn, Zn
A-3……………. Com-3. Cr, Fe, Zn
A-4……………. Com-4. Cr, Cu, Mg, Zn, Mn, Fe

Box-B Supplementation of elements
Sub-boxes:
B-5……………. Ind-5. Cr
B-6……………. Ind-6. Zn
B-7……………. Ind-7. Mg
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 ………….. Sample not supplemented

Com =combination, **Ind**= Individual

For supplementation of required amount of these elements to vegetables, specified volume was taken (Table 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. Fenugreek/Methi (Trigonella-foenum-graceum), Sarson (Brassica-campestris-var-sarson) and (Garlic) (Allium-sativum) were harvested after 66, 41 and 167 days respectively. In case of Fenugreek and Sarson 1st application was applied after 10 days of sowing and 2nd with a gap of 15 days. In case of Garlic 1st application was applied after 30 days of sowing and 2nd with a gap of 45 days.

Table 1: Amount of Fe, Zn, Mn, Cr, Mg, and Cu taken for pplication to experimental plants.

Element	Concentration of element per ml 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

These winter vegetables were watered 14 liters per sub-box once a week. The water was applied very slowly through bucket in intervals to prevent water runoff from the box and to ensure maximum penetration.

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 tape 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 0C until the weight became stable. Samples were then ground with mortar and 0.5g of each sample was wet digested with HNO_3: $HClO_4$ (2:1) for 2-3 hrs on heating mantle[14]. Digested samples were filtered through 0.45 µm pore size Millipore filter and volume was made to 100 ml with distilled water. Minerals concentration was determined on Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer equipped with standard hallow 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 metals. 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 0C [15].Texture was determined by Bouyoucos Hydrometer method using Na_2CO_3 as dispersing agent[16], Organic matter by Walkly and Black method as described by Jackson[17], Lime content ($CaCO_3$) by the acid neutralization method according to Black[18], Potassium (K) by Flame Photometer, Phosphorous (P) by Rapid and sensitive colorimetric method using spectrophotometer. DTPA-Extractable minerals 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[19].

Water analysis

Tap water used for irrigation purpose was analyzed only for: pH, electrical conductivity (EC), chloride as Cl -1, sulphate as SO-4, and minerals by Atomic Absorption Spectrophotometer[20].

Fertilizers and minerals salts applied[21]

Diammonium Phosphate $(NH_4)2\ HPO_4$, Ferrous Sulfate (Sigma) ($Fe\ SO_4.7H_2O$), Zinc Sulfate (Merck) extra pure($Zn\ SO_4.7H_2O$), Manganese Sulphate (Sigma) ($Mn\ SO_4.H_2O$), Chromium (III)-chloride pure crystal RieDel-De-Haen AG. Seelze-Hannover) $[CrCl_2\ (H_2O)4]\ Cl.\ 2H_2O$, Magnesium Sulfate (Merck) extra pure Mg SO4. $7H_2O$ and Copper Sulfate (Sigma) $Cu\ SO_4.5H_2O$.

RESULTS AND DISCUSSION

The chemical analysis and minerals results of soil and water are given in tables 2 to 5.

Soil

The result of the soil texture revealed that it was mainly Clay-loam. 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 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 K2O is required [22].

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 % [23]. 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 [24].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-1, NO2-1 .etc. The soil is non-saline and such soils present no harm to agricultural crops[25].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±0.202and 39.00±0.231mg/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±0.173 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±0.144 mg/Kg at (30-45cm) (Table-4).Thus according to Halvin and Sultan pour, the results indicate that the soil was adequate in Cu, Fe, Mn, and Zn in terms of their concentrations for agricultural crops[19].

Water

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)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-1, NO2-1 .etc. The Chloride content of the water was; 31.14 mg/L. This 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. The amount of sulphate was 30.19 mg/L. where as the recommended limit for sulphate is (250 mg/L) .[26]

***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±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 6 and Figure 1).

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..

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-7 and Figure-2).

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 2: Chemical analysis of soil.

Soil sample Depth	Textural Class			Lime content $CaCO_3$ %	Organic Matter %	P (mg/Kg)	K (mg/Kg)	pH	EC dS/m
	Clay %	Silt %	Sand %						
(0-15cm)	28.8	32.0	39.2	12.66	0.92	7.23	208	7.4	0.10
(15-30 cm)	28.8	28.0	43.2	13.33	1.19	6.66	192	7.3	0.11
(30-45 cm)	28.0	28.4	43.6	13.45	1.18	6.32	185	7.3	0.11
(0-45cm) Average	28.53	29.46	42	13.15	1.10	6.74	195	7.3	0.12

Table 3: 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

Table 4: Minerals in soil (mg/Kg) dry weight basis.

Soil sample Depth	Cr	Zn	Mn	Cu	Mg	Fe
(0-15cm)	2.42±0.006	5.00±0.011	20.69±0.115	6.90±0.023	35.30±0.202	39.00±0.231
(15-30 cm)	2.40±0.006	4.26±0.006	19.54±0.087	5.40±0.006	33.82±0.115	38.92±0.173
(30-45 cm)	2.44±0.011	4.50±0.017	19.37±0.069	5.20±0.006	32.86±0.086	38.46±0.144

Table 5: Minerals in water (mg/L).

Sample	Cr	Zn	Mn	Cu	Mg	Fe
Water	0.06±0.000	0.07±0.006	0.02±0.000	0.05±0.000	21.81±0.040	2.39±0.011

Table 6: Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) dry weight basis. *Trigonella-foenum-graceum.* (Fenugreek/ Methi)

Sub-boxes	Concentration(mg/Kg) Dry Weight Basis					
	Cr	Zn	Mn	Cu	Mg	Fe
A-1	22±0.173	66±0.578	64±0.578	10±0.104	2100±6.936	1776±15.02
A-2	28±0.144	94±0.462	74±0.289	8±0.028	2280±6.936	1862±15.02
A-3	28±0.121	96±0.578	64±0.462	10±0.092	1980±8.092	2092±11.56
A-4	22±0.202	162±1.156	76±0.462	10±0.104	3070±5.780	1882±11.56
B-5	34±0.231	64±0.462	68±0.346	8±0.023	3214±6.936	1664±21.96
B-6	16±0.098	90±0.693	68±0.346	12±0.086	3180±6.936	1568±13.87
B-7	14±0.086	66±0.635	68±0.404	8±0.040	3264±24.27	1670±26.58
B-8	12±0.057	94±0.520	62±0.289	10±0.086	3104±11.56	1404±11.56
C-9	32±0.057	62±0.520	44±0.173	8±0.040	2726±13.87	1032±9.248
C-10	18±0.075	96±0.462	54±0.231	8±0.034	2864±15.02	1236±12.71
C-11	16±0.092	64±0.404	60±0.225	14±0.104	3176±6.936	1866±33.52
C-12	24±0.115	68±0.462	66±0.323	14±0.115	3008±19.65	1572±11.56

C-12= sample without supplementation, Mean±SE

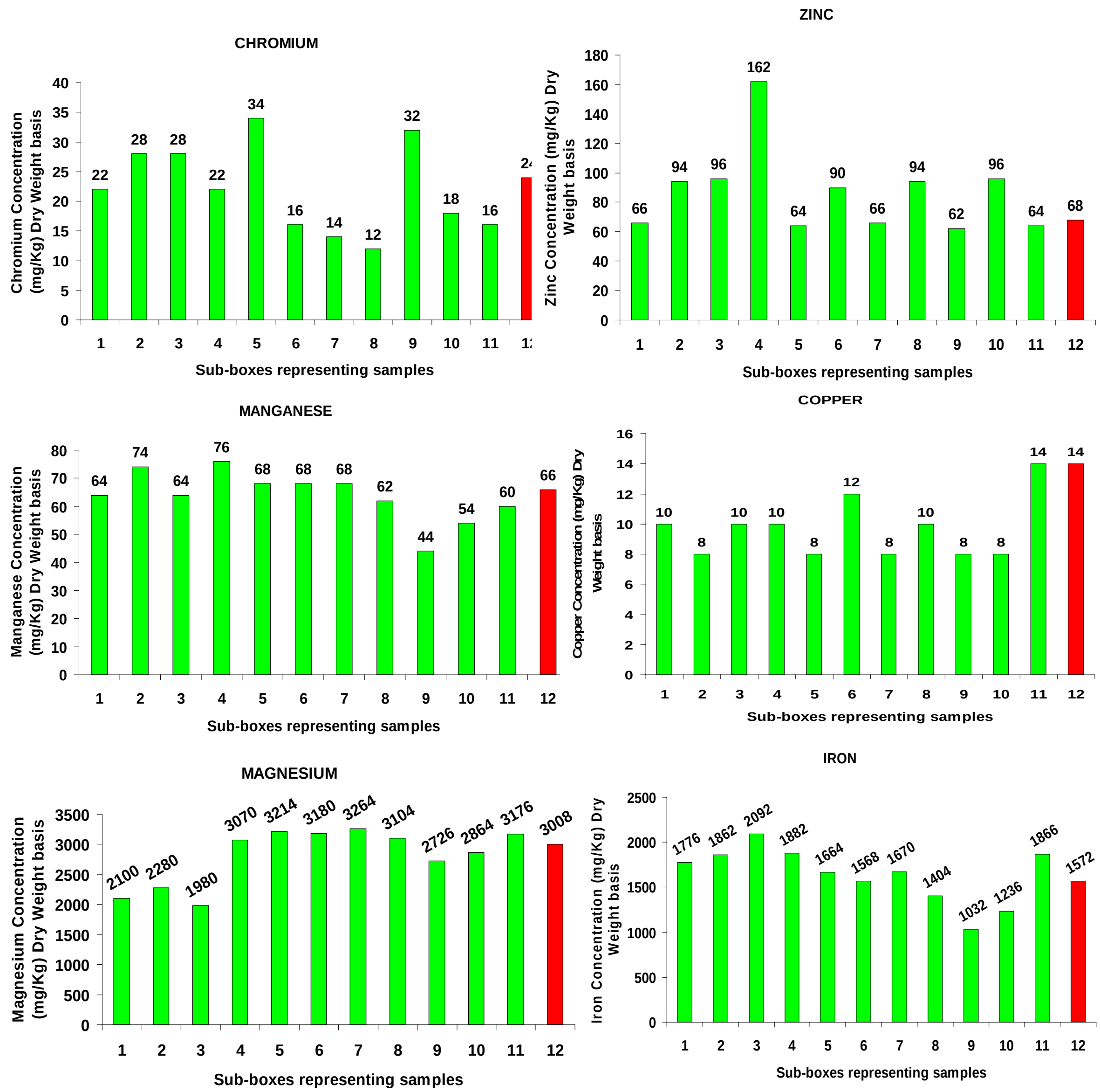

Figure 1: *Trigonella-foenum-graceum.* (Fenugreek) Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg dry weight basis.

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 8 and Figure 3).

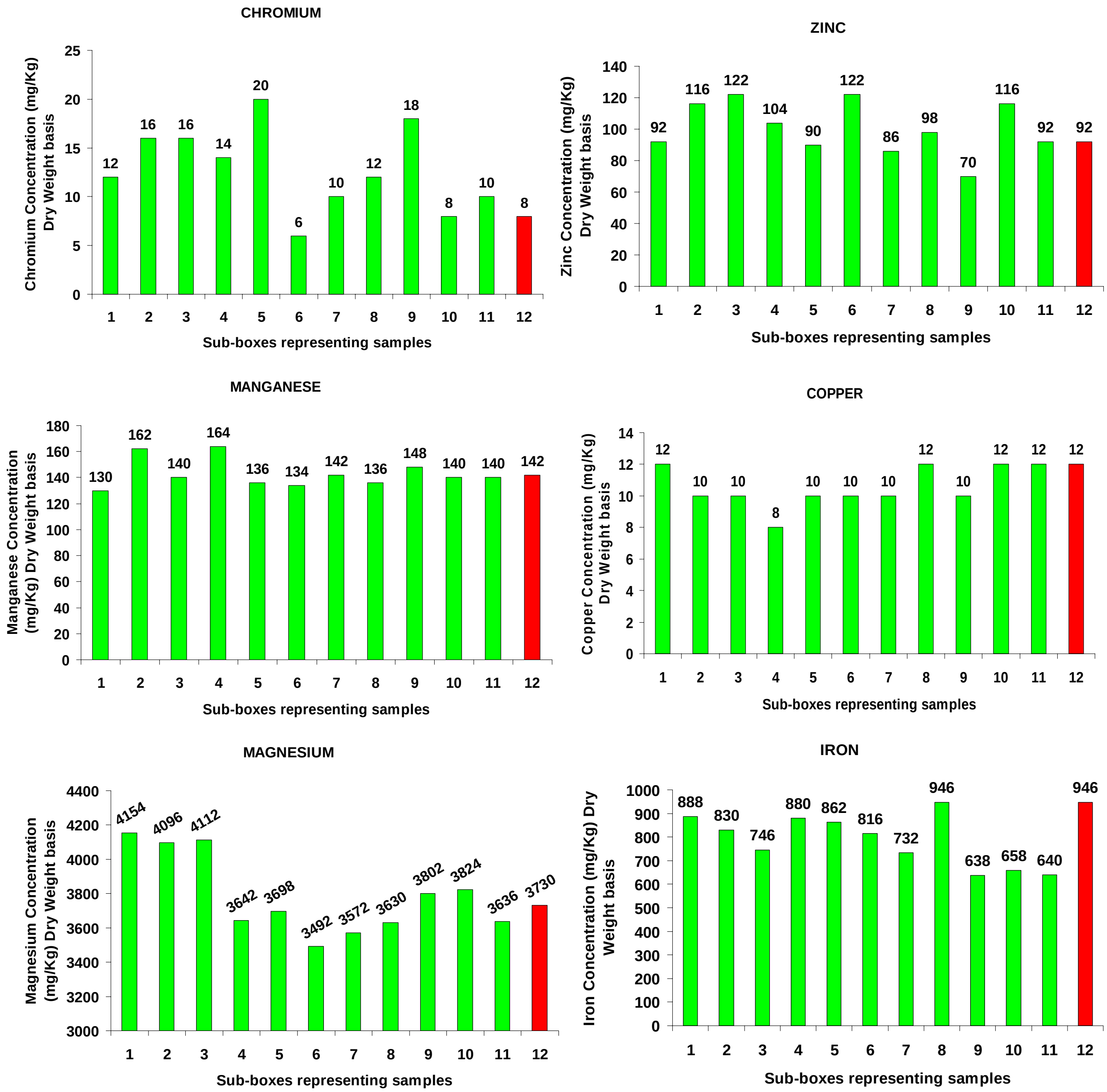

Figure 2: *Brassica-campestris-var-sarson* (Sarson) Concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg dry weight basis.

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 7: *Brassica-campestris-var-sarson*.(Sarson) Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) dry weight basis**.**

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis					
	Cr	**Zn**	**Mn**	**Cu**	**Mg**	**Fe**
A-1	12±0.057	92±0.462	130±0.578	12±0.092	4154±9.248	888±2.312
A-2	16±0.098	116±0.867	162±0.578	10±0.046	4096±12.71	830±2.890
A-3	16±0.092	122±0.924	140±0.924	10±0.046	4112±6.936	746±1.156
A-4	14±0.086	104±0.635	164±0.867	8±0.023	3642±6.936	880±2.890
B-5	20±0.121	90±0.693	136±0.693	10±0.034	3698±15.02	862±1.734
B-6	6±0.023	122±1.040	134±1.156	10±0.040	3492±10.40	816±1.734
B-7	10±0.034	86±0.462	142±0.924	10±0.040	3572±5.780	732±3.468
B-8	12±0.069	98±0.520	136±0.722	12±0.098	3630±20.80	946±1.156
C-9	18±0.075	70±0.404	148±1.156	10±0.104	3802±10.40	638±1.734
C-10	8±0.040	116±0.809	140±0.867	12±0.092	3824±10.40	658±2.890
C-11	10±0.028	92±0.578	140±0.867	12±0.104	3636±16.18	640±3.468
C-12	8±0.040	92±0.578	142±0.809	12±0.104	3730±10.40	946±1.156

C-12= sample without supplementation, Mean±SE

Table 8: *Allium-sativum* (garlic)Concentration of Cr,Zn,Mn,Cu,Mg and Fe(mg/Kg) dry weight basis

Sub-boxes	Concentration (mg/Kg) Dry Weight Basis					
	Cr	**Zn**	**Mn**	**Cu**	**Mg**	**Fe**
A-1	12±0.057	68±0.578	20±0.173	14±0.080	788±1.734	146±0.936
A-2	12±0.075	100±0.867	18±0.080	10±0.040	692±2.312	158±0.809
A-3	14±0.104	104±0.693	20±0.104	10±0.046	630±1.734	160±0.722
A-4	12±0.080	100±0.693	24±0.173	14±0.069	694±1.734	146±0.867
B-5	22±0.173	88±0.578	22±0.115	14±0.069	870±2.890	160±1.156
B-6	12±0.069	120±1.040	22±0.115	14±0.080	768±2.312	140±0.924
B-7	10±0.046	84±0.462	18±0.086	10±0.046	758±1.156	148±0.635
B-8	10±0.046	98±0.462	20±0.098	10±0.040	884±2.312	140±1.040
C-9	20±0.115	72±0.404	22±0.104	12±0.046	898±2.890	116±0.809
C-10	10±0.040	116±0.924	20±0.104	12±0.057	902±1.734	138±0.838
C-11	12±0.063	88±0.635	22±0.104	12±0.057	896±2.890	144±0.612
C-12	8±0.028	92±0.520	20±0.098	10±0.046	786±1.156	150±0.693

C-12= sample without supplementation, Mean±SE.

From results of chromium and zinc of these winter 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[27]. 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.

Chromium (Cr) deficiency is a causative factor for NIDDM (Non-insulin dependent diabetes mellitus), producing symptoms including fasting hyperglycemia, impaired glucose tolerance, decreased insulin binding and receptor number, decreased HDL, and increased total cholesterol and triglycerides[28]. 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[29].

Zinc plays a role in the synthesis of insulin by pancreatic beta cells[30-31] and in the action of insulin at the cellular level[32].

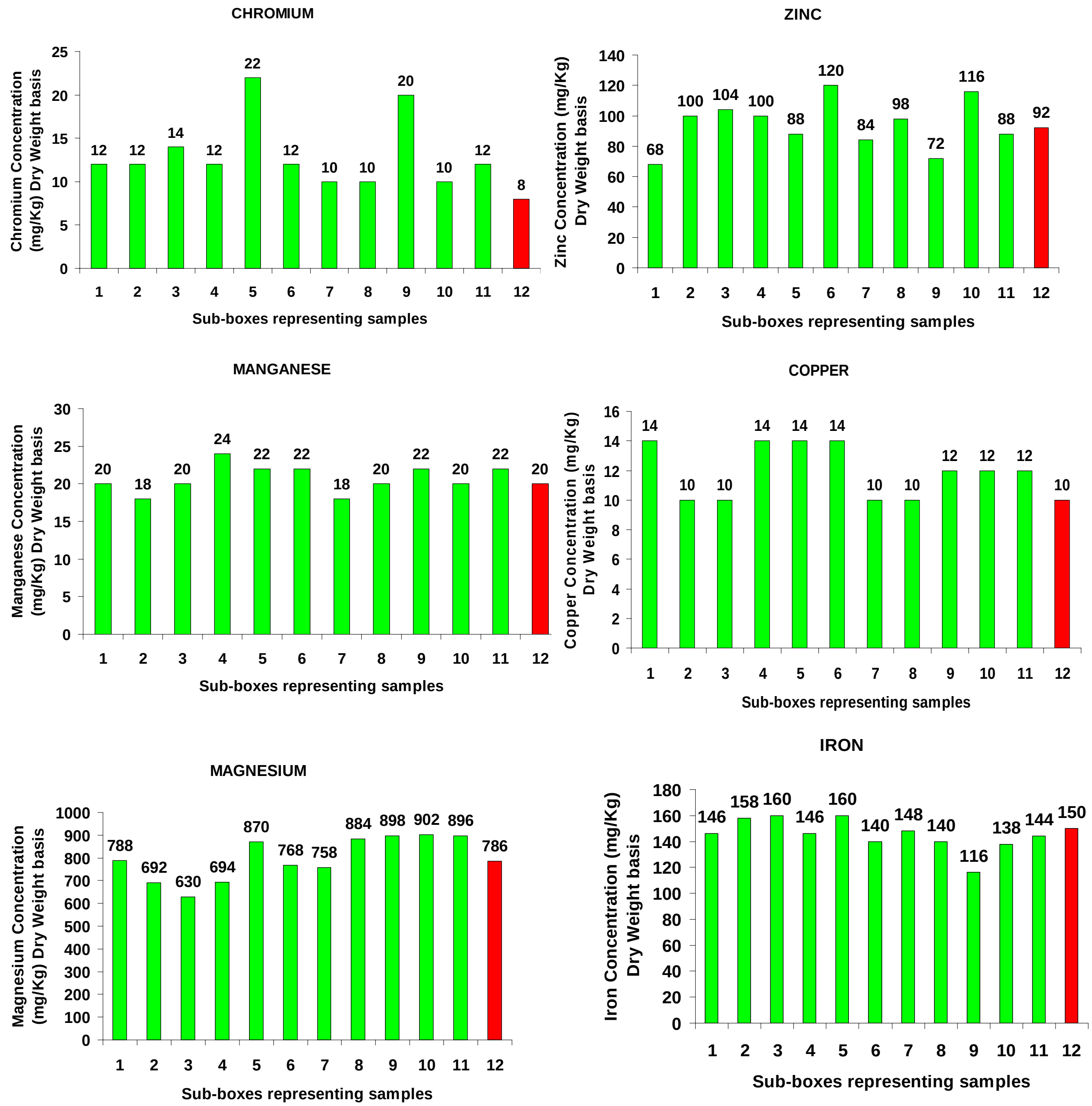

Figure 3: *Allium-sativum*.(Garlic) concentration of Cr, Zn, Mn, Cu, Mg and Fe mg/Kg dry weight basis

The results obtained 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[32]. 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[33].

Copper, manganese and iron were supplemented in the form of combinations (1, 4); (2, 4); (3, 4) respectively. On the basis of our results combination (1) is best for copper and combination (4) for manganese. 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). Two male volunteers who consumed a controlled intake of 0.7-0.8 mg of copper per day for 5-6 months had increased[34] glucose levels during a glucose tolerance test. These levels returned toward normal after adequate amounts of copper were restored to the diet[35]. Manganese (Mn) plays an important role in a number of physiologic processes as a constituent of some enzymes and an activator of other enzymes[36].

As iron was 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. 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 [37-38].

CONCLUSION

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. This in turn could play important role in diabetes control and also in the elimination of 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.

REFERENCES

1. Loomis RS and Conner DJ. Crop Ecology: Productivity and Management in Agricultural Systems. Cambridge U. Press, Cambridge, 1992.
2. Epstein, E. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci.,* USA. 1994; 91: 11-17.
3. Marschner H. "Mineral nutrition of higher plants." San Diego: Academic Press, 1995.
4. Kochian LV. Mechanisms of micronutrient uptake and translocation in plants. In Mordvedt JJ, Cox FR, Shuman LM, Welch.RM (eds):"Micronutrients in Agriculture," 2nd ed. Madison WI: Soil Science Society of America, 1991; pp 229-296.
5. Maathuis FJM and Sanders D. Plasma membrane transport in context-making sense out of complexity. *Curr. Opin. Plant Biol.*, 2002; 2: 236-243.
6. Nielsen FH. Other trace elements. In Ziegler EE, Filer Jr LJ (eds):"Present Knowledge in Nutrition," 7th ed. Washington, DC: International Life Sciences Institute, 1996; pp 353-377.
7. Kabata-Pendias A and Pendias H."Trace Elements in Soils and Plants," 2nd ed. Boca Raton FL: CRC 1992.
8. Fairweather-Tait SJ. Bioavailability of dietary minerals. *Biochemical Society Transactions,* 1996; 24: 775-780.
9. Banuelos GS, Ajwa HA, Terry NE and Zayed A. Phytoremediation of Se laden soils: A new technology. *J Soil Water Conserv.,* 1997; 52: 426-430.
10. Shils ME. Magnesium. In: Shils M, Olson JA, Shike M, Ross AC, eds. Nutrition in Health and Disease. 9th ed. Baltimore: Williams & Wilkins, 1999; 169-192.
11. Wauchope RD. Selenium and arsenic levels in soybeans from different production regions of the United States. *J. Agric. Food Chem*., 1978; 26: 226-228.
12. Lappalainen R. The concentration of zinc and Mg in human enamel and dentine related to age their concentration in the soil. *Arch. Oral Biol.,*1981; 26: 1-6.
13. Rabinovitz H. Effect of chromium supplementation on blood glucose and lipid levels in type 2 diabetes mellitus elderly patients. *Int. J. Vitam. Nutr. Res.,* 2004; 74: 178-182.
14. AO A C. Official Methods of Analysis 14th Ed. Sidney Williams. Association of Official Chemists, Inc. Virginia, USA, 1984.
15. Richard LA. Diagnosis and improvement of saline and alkali soils, U.S.D.A. Hand Book, 1960.
16. Kochler FE, Moddie CD and McNeal BL. Laboratory Manual for Soil Fertility, Washington State Univ: Pullma Washington, 1984.
17. Jackson ML. Soil Chemical Analysis. Prentice Hall Inc. Engle-Wood Cliffs, NJ, 1958; pp 372.
18. Black CA. Methods of Soil Analysis Part-II Soc. Argon. Inc. Publ. Madison Wisconism, USA, 1965.
19. Halvin JL and Sultanpour PN. *Soil Sci. Soc. Amer, J*., 1981; 45: 70.
20. APHA and AWWA. "Standards Methods for the Examination of Water and Wastewater," 20th ed., American Public Health Inc. New York, 1998.
21. Alam SM and Raza S. "Micronutrient Fertlizers" *Pak. J. Biol. Sci.,*. 2001; 4: 1446-1450.
22. Rehman N, Azim K and Ilyas M. A Laboratory Manual for Soil and Water Testing Methods. Barani Agri. Research Station, Kohat, 2001.
23. Bhatti AV. "Irrigated Soils and Water Management in NWFP, Deptt. of Water Management NWFP Agri. University Peshawar, 1997.
24. Fulter WH. "Movement of Selected metals, asbestos and cyanidein soil: application to waste disposal problem" EPA-6000/2-77-020 solid and Hazardous wastes Res. Div. USA, 1977; pp 243.
25. Maas EV and Hoffman GJ. Crop Salt Tolerance Current Assessment" *J. Irrig. Drainage Div.*, ASCE, 1986; 103-115.
26. USEPA. "Proposed guidelines for the Health Risk Assessment of Chemical Mixtures" Fed. Reg. 50 (6) Washington, DC, 1985.
27. Zurayk R, Sukkariyah B, Baalbaki R and Ghanem DA. Chromium phytoaccumulation from solution by selected hydrophytes. *Int. J. of Phytoremediation,* 2001; 2: 335-350.
28. Anderson RA. Chromium, glucose tolerance, diabetes and lipid metabolism. *J Advan Med.,* 1995; 8: 37-50.
29. Stearns DM, Wise JP, Patierno SR and Wetterhahn KE. Chromium (III) picolinate produces chromosome damage in Chinese hamster ovary cells. *Faseb J*., 1995; 9: 1643-1648.
30. Emdin SO. Role of zinc in insulin biosynthesis. Some possible zinc-insulin interactions in the pancreatic B-cell. *Diabetologia*. 19, (1980)174-182.
31. Herington AC. Effect of zinc on insulin binding to rat adipocytes and hepatic membranes and to human placental membranes and IM9 lymphocytes. *Horm. Metab. Res*., 1985; 17: 328-332.
32. Meyer KA, Kushi LH, Jacobs DR, Slavin J, Sellers TA and Folsom AR. Carbohydrates, dietary fiber, and incident type-2 diabetes in older women. *Am. J. Clin. Nutr*., 2000; 71: 921-930.

33. Manohar V. Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. *Diabetes Obes. Metab*., 2002; 4: 43-48.
34. Klevay LM. Diminished glucose tolerance in two men due to a diet low in copper. *Am. J. Clin. Nutr*., 1983; 37: 717.
35. Nielsen FH. Ultratrace minerals. In: Shils M, Olson JA, Shike M, Ross AC, eds. Nutrition in Health and Disease. 9th ed. Baltimore: Williams and Wilkins, 1999; pp 283-303.
36. Beard JL, Dawson HD. Iron. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential minerals. New York: Marcel Dekker, Inc., 1997; pp 275-334.
37. Fairbanks VF. Iron in Medicine and Nutrition. In: Shils M, Olson JA, Shike M, Ross AC, eds. Nutrition in Health and Disease. 9th ed. Baltimore: Williams and Wilkins, 1999; pp 223-239.