Effect of Season and Soil Concentration on the Uptake of Minerals in *Spinacia Oleracea*

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Summary: To study the effect of season and soil concentration on the uptake of minerals in *Spinacia oleracea*, it was grown on minerals chromium (Cr), zinc (Zn), manganese (Mn), copper (Cu), magnesium (Mg) and iron (Fe) supplemented soil both in winter and summer seasons, respectively. For this purpose a plot was prepared having 12 sub-boxes. *Spinacia oleracea* was grown in these boxes and supplemented either with individual element or combination of elements except in box-12 for comparison. Before sowing seeds, soil samples were taken and analyzed for pH, electrical conductivity (EC), texture, lime content ($CaCO_3$), potassium, phosphorus and concentrations of extractable heavy metals (Cr, Zn, Mn, Cu, Mg and Fe). Irrigation water was also analyzed for pH, electrical conductivity (EC), chloride, sulphate and Cr, Zn, Mn, Cu, Mg and Fe concentrations. After harvesting vegetable samples were dried, acid digested and analyzed for Cr, Mn, Zn, Cu, Fe and Mg on Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer. Chromium, zinc, copper, magnesium and iron contents were increased in all summer vegetables. The increase was quite marked suggesting that summer season is more suitable for enhancing the contents of these minerals than winter. The present study was an attempt to see whether it is possible to enhance the level of nutritionally and therapeutically important minerals in vegetables to reduce their serving size. The study revealed that it is possible.

Introduction

Plants are known to accumulate various chemicals from their environment [1, 2]. Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well-being [3], 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 [4]. Climate may effect the concentration in plant as shown by the deficiency of Zn in cool and wet season which is associated with insufficient solubility of Zn in soil [5]. Metals accumulation in plant depends on plant species, growth stages, type of soil, metals, soil conditions, weather, and environment [6]. Heavy metal contamination of vegetables may also occur due to irrigation with contaminated water [7]. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils [8].

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 [9].

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 [10], and thus their incorporation into plant tissues is of dietary relevance.

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 [11].

Minerals work in combination with each other and with other nutrients, so imbalances of any mineral can cause health problems. Too little use of any essential mineral can lead to deficiency diseases and too much use of any mineral can be toxic [12].

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

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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. In the present study the main idea was to change the soil concentration with selected nutritionally important minerals, and to see the extent of their uptake in *Spinacia oleracea* with respect to season.

Results and Discussion

The chemical analysis and minerals results of soil and water are given in Table 1-4 while Cr, Zn, Mn, Cu, Mg, and Fe concentrations of *Spinacia oleracea (Spinach)* in winter and summer are given in Table-5 and 6, respectively.

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% (0-15 cm); 1.19% (15-30 cm), and 1.18% (30-45 cm) 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 60 kg/ha nitrogen (N) is recommended [13] (Table-1).

Phosphorus results obtained for different depths were in the order of; 7.23 mg/Kg (0-15 cm); 6.66 mg/Kg (15-30 cm), and 6.32 mg/Kg (30-45 cm) with an average of 6.74 mg/Kg. Likewise, potassium results for different depths were; 208 mg/Kg (0-15 cm); 192 mg/Kg (15-30 cm), and 185 mg/Kg (30-45 cm) with an average of 195 mg/Kg. If the soil's test phosphorus (P) level is 5-10 mg/Kg then phosphorus (P_2O_5) 60 kg/ha is recommended. Similarly if the soil test Potassium (K) level is above 150 then no K_2O is required [13] (Table-1).

The lime content ($CaCO_3$) at different depths found was: 12.66% (0-15 cm); 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 from 3.85 to 24.2% [14]. pH of soil at different depth level was; 7.4 (0-15 cm); 7.3 (15-30 cm), and 7.3 (30-45 cm), 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 [15] (Table-1).

The electrical conductivity (EC) at different depths was in the order of 0.10 dsm^{-1} (0-15 cm); 0.11 dsm^{-1} (15-30 cm), and 0.11 dsm^{-1}(30-45 cm) 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}, NO_2^{-1} *etc*. The soil is non-saline, and such soils present no harm to agricultural crops [16] (Table-1).

The concentrations of Cr, Zn, Mn, Cu, Mg, and Fe found in soil on dry weight basis at different depths were: 2.42 ± 0.01, 5 ± 0.02, 20.69 ± 0.20, 6.90 ± 0.04, 35.30 ± 0.35and 39.00 ± 0.40mg/kg at (0-15 cm) 2.40 ± 0.01, 4.26 ± 0.01, 19.54 ± 0.15, 5.40 ± 0.01, 33.82 ± 0.20, and 38.92 ± 0.30 mg/Kg at (15-30 cm); 2.44 ± 0.02, 4.50 ± 0.03, 19.37 ± 0.12, 5.20 ± 0.01, 32.86 ± 0.15, and 38.46 ± 0.25 mg/Kg at (30-45 cm). (Table-2). Thus the soil was adequate in Cu, Fe, Mn, and Zn in terms of their concentrations for agricultural crops [17].

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}, NO_2^{-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 whereas the recommended limit for sulphate is (250 mg/L) (Table-3) [18]. The concentrations of Cr, Zn, Mn, Cu, Mg, and Fe found in water were: 0.06, 0.07 ± 0.01, 0.02, 0.05, 21.81 ± 0.07 and 2.39 ± 0.02, respectively. (Table-4).

Table-1: Chemical Analysis of Soil.

Soil sample Depth	Textural Class			Lime content	Organic	P	K	pH	EC
	Clay %	Silt %	Sand %	$CaCO_3$ %	Matter %	(mg/Kg)	(mg/Kg)		dS/m
(0-15 cm)	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
(0-45 cm) Average	28.53	29.46	42	13.15	1.10	6.74	195	7.3	0.12

Table-2: Minerals in Soil (mg/Kg) Dry Weight Basis.

Soil sample	Cr	Zn	Mn	Cu	Mg	Fe
Depth (0-15 cm)	2.42 ± 0.01*	5.00 ± 0.02	20.69 ± 0.20	6.90 ± 0.04	35.30 ± 0.35	39.00 ± 0.40
Depth (15-30 cm)	2.40 ± 0.01	4.26 ± 0.01	19.54 ± 0.15	5.40 ± 0.01	33.82 ± 0.20	38.92 ± 0.30
Depth (30-45 cm)	2.44 ± 0.02	4.50 ± 0.03	19.37 ± 0.12	5.20 ± 0.01	32.86 ± 0.15	38.46 ± 0.25

*Average of triplicate determination ± S.D (Standard Deviation)

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 Water (mg/L).

Sample	Cr	Zn	Mn	Cu	Mg	Fe
Water	0.06 ± 0.00*	0.07 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	21.81 ± 0.07	2.39 ± 0.02

*Average of triplicate determination ± S.D (Standard Deviation)

Spinacia oleracea (Spinach) Winter

The concentration of Cr, Zn, Mn, Cu, Mg, and Fe in the sample (C-12) not supplemented observed was (22 ± 0.30, 130 ± 1.50, 164 ± 2.00, 18 ± 0.10, 14740 ± 16, and 3124 ± 18 with a maximum concentration of Cr, Zn, Mn, Mg, and Fe 42 ± 0.20, 196 ± 3.00, 196 ± 2.00, 16460 ± 16, and 3498 ± 14) mg/Kg on dry weight basis in samples (B-5), (A-4), (A-4), (C-9), and (A-4) respectively. Only concentration of Cu did not increase or decrease with respect to the sample not supplemented. The total increase of Cr, Zn, Mn, Mg, and Fe recorded was (20, 66, 32, 1720, and 374) mg/Kg dry weight basis with respect to sample (C-12). While in samples (B-6), (B-7), (B-7), (B-7), (B-7), and (C-10) minimum concentration of Cr, Zn, Mn, Cu, Mg, and Fe (10 ± 0.07, 110 ± 1.60, 110 ± 0.80, 8 ± 0.08, 9800 ± 20and 2074 ± 12with total decrease of 12, 20, 54, 10, 4940, and 1050) mg/Kg dry weight basis was observed (Table-5).

Table-5: Concentration of Cr,Zn,Mn,Cu,Mg, and Fe(mg/Kg) Dry Weight Basis (*Spinacia oleracea* Winter).

Sub-boxes Samples	Concentration (mg/Kg) Dry Weight Basis					
	Cr	Zn	Mn	Cu	Mg	Fe
A-1	24 ± 0.35*	126 ± 1.00	156 ± 1.40	16 ± 0.18	14560 ± 52	3120 ± 18
A-2	18 ± 0.18	142 ± 2.00	108 ± 1.20	14 ± 0.20	11440 ± 32	2310 ± 14
A-3	18 ± 0.16	178 ± 4.00	168 ± 1.60	12 ± 0.16	13620 ± 44	2742 ± 28
A-4	16 ± 0.16	196 ± 3.00	196 ± 2.00	16 ± 0.17	15620 ± 14	3498 ± 14
B-5	42 ± 0.20	122 ± 1.80	136 ± 1.40	12 ± 0.15	15080 ± 10	2608 ± 18
B-6	10 ± 0.07	140 ± 1.60	118 ± 1.60	12 ± 0.16	14020 ± 18	2344 ± 52
B-7	16 ± 0.20	110 ± 1.60	110 ± 0.80	8 ± 0.08	9800 ± 20	2302 ± 14
B-8	30 ± 0.35	182 ± 1.80	140 ± 1.40	14 ± 0.18	14620 ± 14	2604 ± 26
C-9	40 ± 0.10	124 ± 1.20	140 ± 1.60	14 ± 0.18	16460 ± 16	2922 ± 16
C-10	16 ± 0.16	146 ± 2.00	132 ± 1.50	16 ± 0.17	16300 ± 38	2074 ± 12
C-11	18 ± 0.18	126 ± 1.20	160 ± 2.00	16 ± 0.17	15820 ± 32	3024 ± 22
C-12	22 ± 0.30	130 ± 1.50	164 ± 2.00	18 ± 0.10	14740 ± 16	3124 ± 18

*Average of triplicate determination ± S.D (Standard Deviation)
C-12= Sample not supplemented with minerals

Spinacia oleracea (Spinach) Summer

The concentration of Cr, Zn, Mn, Cu, Mg, and Fe in the sample (C-12) not supplemented observed were (26 ± 0.20, 128 ± 1.20, 734 ± 6.00, 16 ± 0.17, 14120 ± 22, and 2852 ± 18 with a maximum concentration of Cr, Zn, Cu, Mg, and Fe (116 ± 1.60, 364 ± 3.00, 536 ± 6.00, 22920 ± 28, and 3994 ± 16) mg/Kg on dry weight basis in samples (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 with respect to sample (C-12). While in samples (C-11), (A-1), (C-9), (B-7), (B-5) and (B-5) minimum concentration of Cr, Zn, Mn, Cu, Mg, and Fe (18 ± 0.17, 100 ± 1.15, 216 ± 5.00, 14 ± 0.14, 8160 ± 14and 2626 ± 40 with total decrease of 8, 264, 518, 2, 5960, and 226) mg/Kg dry weight basis was observed (Table-6).

Table-6: Concentration of Cr,Zn,Mn,Cu,Mg, and Fe(mg/Kg) Dry Weight Basis (*Spinacia oleracea* Summer).

Sub-boxes Samples	Concentration (mg/Kg) Dry Weight Basis					
	Cr	Zn	Mn	Cu	Mg	Fe
A-1	40 ± 0.50*	100 ± 1.15	284 ± 8.00	20 ± 0.20	22920 ± 28	2814 ± 16
A-2	38 ± 0.40	138 ± 1.10	416 ± 2.00	18 ± 0.19	15980 ± 12	2814 ± 54
A-3	36 ± 0.25	150 ± 1.80	278 ± 2.00	14 ± 0.15	17100 ± 18	3546 ± 66
A-4	48 ± 0.30	142 ± 1.20	536 ± 6.00	24 ± 0.30	18540 ± 18	3994 ± 16
B-5	116 ± 1.60	128 ± 1.10	344 ± 0.00	16 ± 0.17	8160 ± 14	2626 ± 40
B-6	24 ± 0.25	364 ± 3.00	282 ± 6.00	16 ± 0.17	12700 ± 14	2842 ± 14
B-7	20 ± 0.21	124 ± 1.20	336 ± 2.00	14 ± 0.14	16780 ± 52	2806 ± 32
B-8	92 ± 1.20	150 ± 1.60	292 ± 6.00	14 ± 0.15	18060 ± 16	3018 ± 12
C-9	112 ± 1.20	122 ± 1.00	216 ± 5.00	18 ± 0.17	14360 ± 12	2640 ± 14
C-10	20 ± 0.20	360 ± 3.50	300 ± 7.00	14 ± 0.15	10860 ± 26	2672 ± 12
C-11	18 ± 0.17	120 ± 1.00	346 ± 8.00	18 ± 0.18	14180 ± 26	2736 ± 24
C-12	26 ± 0.20	128 ± 1.20	734 ± 6.00	16 ± 0.17	14120 ± 22	2852 ± 18

*Average of triplicate determination ± S.D (Standard Deviation)
C-12= Sample not supplemented with minerals

As shown in Fig. 1 both to the winter, and summer season samples chromium was applied in the form of combinations (1, 2, 3, 4, 8), and individually (5, 9). In case of winter season samples, chromium concentration was increased in samples A-1 (40 ± 0.50 mg/Kg), B-8 (30 ± 0.35 mg/Kg), B-5 (42 ± 0.20 mg/Kg), C-8 (30 ± 0.35 mg/Kg), and C-9 (40 ± 0.10 mg/Kg) with a marked increase in sample B-5 (42 ± 0.20), and decreased in samples A-2 (18 ± 0.18 mg/Kg), A-3 (18 ± 0.16 mg/Kg), A-4 (16 ± 0.16 mg/Kg). Chromium content was decreased where

magnesium, and zinc was applied alone. In case of summer season samples increase in chromium content was occurred in all samples with marked increase in sample B-5 (116 ± 1.60 mg/Kg). Chromium content was decreased where magnesium and zinc were applied alone. The chromium uptake by plants has been found to be positively correlated to chromium application by many workers [19-22].

As shown in Fig. 2 both to the winter, and summer season samples, zinc was applied in the form of combinations (2, 3, 4, 8), and individually (6, 10). In case of winter season samples increase was observed in all cases. Zinc content decreased, where chromium and zinc were applied alone. Marked increase was observed in sample A-4 (196 ± 3.00 mg/Kg). In case of summer season samples zinc content was increased in all samples with marked increase in sample B-6 (364 ± 3.00 mg/Kg). Zinc content decreased where magnesium, and chromium were applied alone, and in sample A-1 (100 ± 1.15 mg/Kg). In case of chromium supplementation no change occurred in sample B-5 (128 ± 1.10 mg/Kg). Zinc concentration in wheat grown with the aid of Zn-containing fertilizers was about twice that of wheat grown on the same soils without Zn applications [23].

As shown in Fig. 3 both to the winter, and summer season samples magnesium was applied in the form of combinations (1, 4, 8), and individually (7, 11). In case of winter season samples decrease was occurred in samples A-1 (14560 ± 52 mg/Kg), B-8 (14620 ± 14 mg/Kg), and B-7(9800 ± 20 mg/Kg), while increased concentration in samples A-4 (15620 ± 14 mg/Kg), and C-11 (15820 ± 32 mg/Kg).Marked increase was recorded in sample C-9 (16460 ± 16 mg/Kg) in case of chromium supplementation. In case of summer season samples magnesium concentration was increased in all samples. Marked increase occurred in samples A-1 (22920 ± 28 mg/Kg). The concentration was decreased in chromium, and zinc supplemented samples with only exception of increase in sample C-9 (14360 ± 12 mg/Kg).Magnesium concentration generally decline as the plant matures [24].

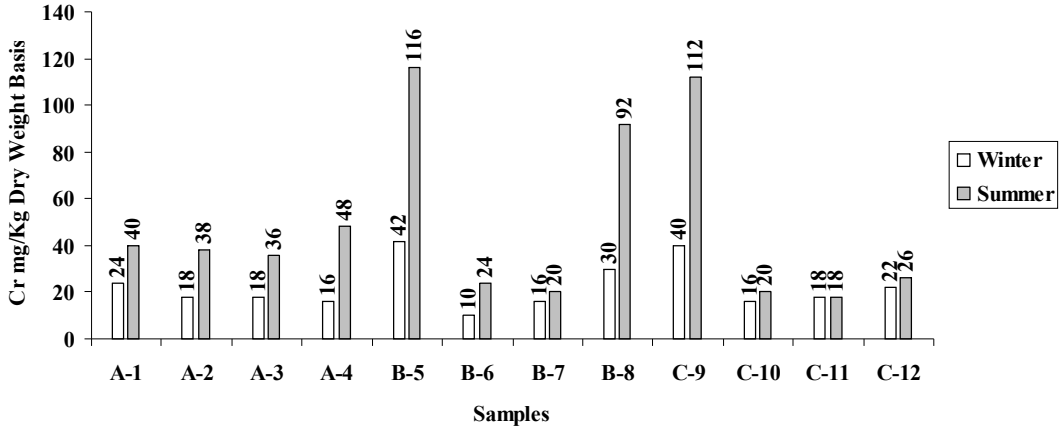

Fig. 1: *Spinacia oleracea* (spinach) concentration of Cr mg/Kg dry weight basis.

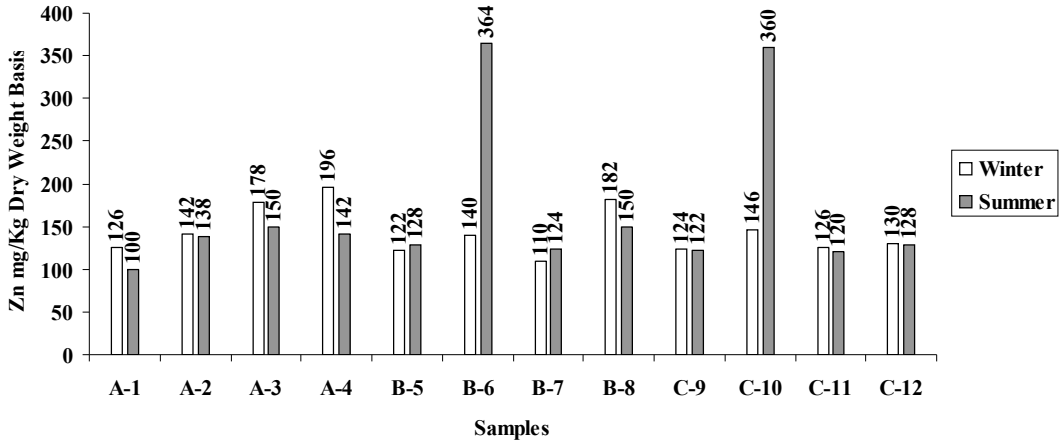

Fig. 2: *Spinacia oleracea* (spinach) concentration of Zn mg/Kg dry weight basis.

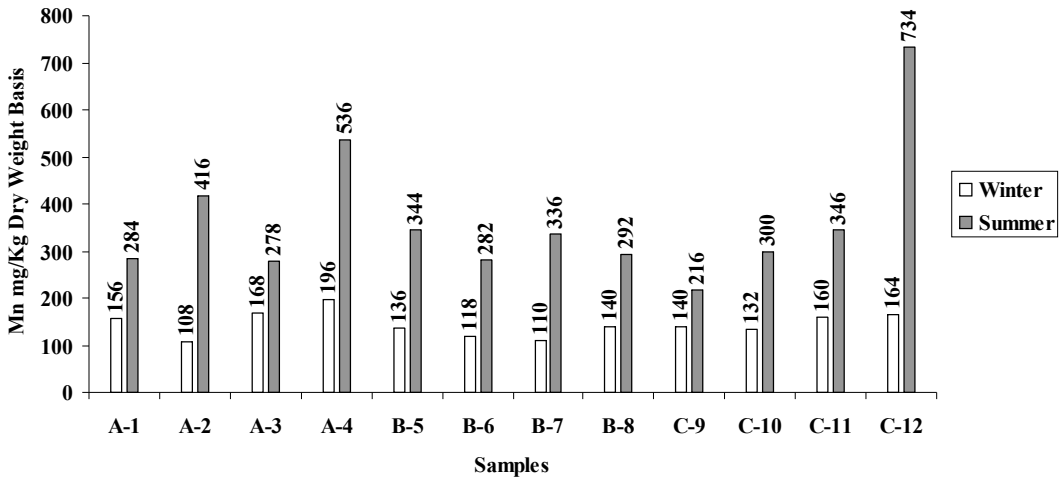

Fig. 3: *Spinacia oleracea* (spinach) concentration of Mn mg/Kg dry weight basis.

As shown in Fig. 4-6, copper, manganese, and iron were applied in combinations (1, 4), (2, 4), and (3, 4) respectively. In case of winter season samples, copper content was decreased in both cases, while manganese, and iron content was increased in sample A-4 (196 ± 2.00 mg/Kg, 3498 ± 14 mg/Kg), and decreased in samples A-2 (108 ± 1.20 mg/Kg), A-3 (2742 ± 28 mg/Kg), respectively. In case of summer season samples, increase in concentration of copper, and iron was occurred in all samples with marked increase in samples A-4 (24 ± 0.30 mg/Kg), and sample A-4 (3994 ± 16 mg/Kg), respectively. Manganese content was decreased in both samples.

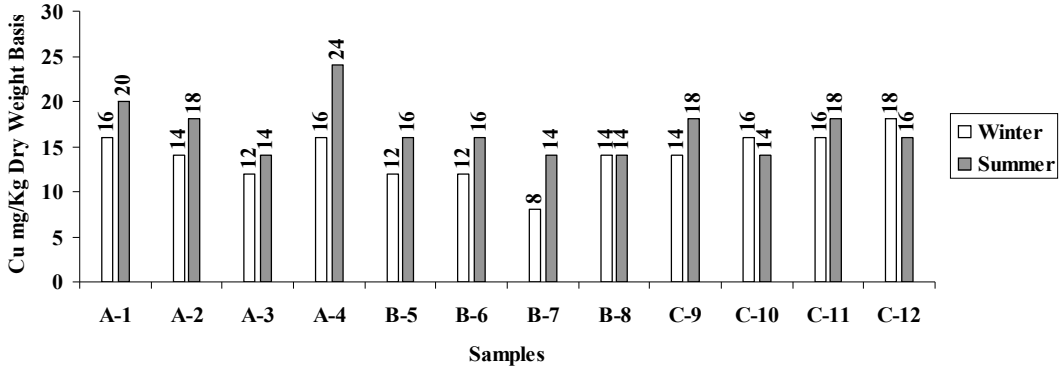

Fig. 4: *Spinacia oleracea* (spinach) concentration of Cu mg/Kg dry weight basis.

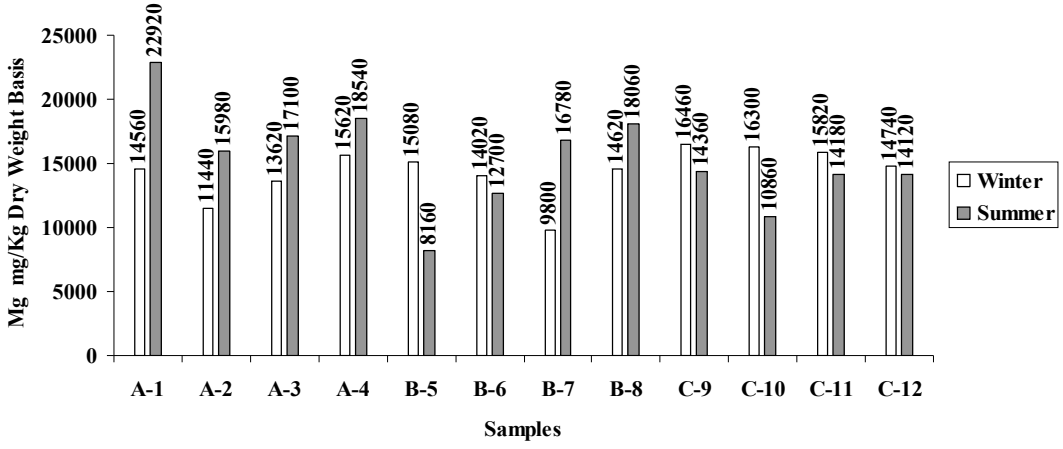

Fig. 5: *Spinacia oleracea* (spinach) concentration of Mg mg/Kg dry weight basis.

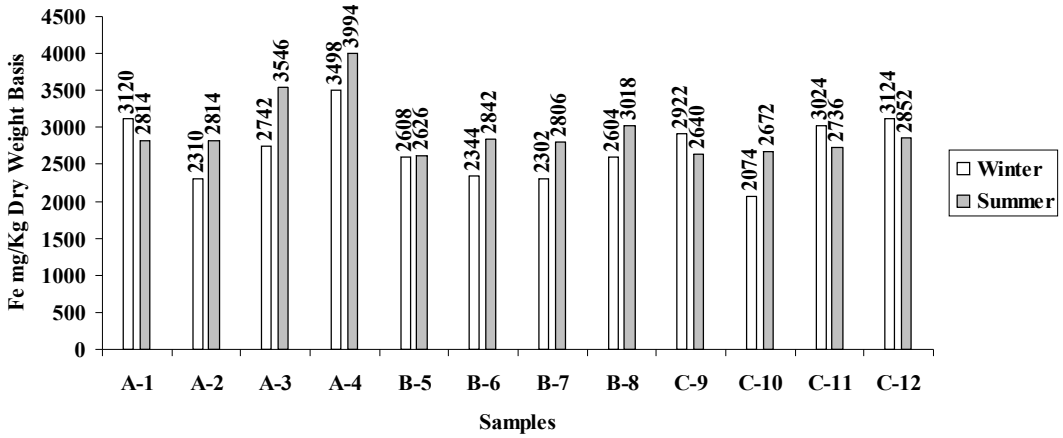

Fig. 6: *Spinacia oleracea* (spinach) concentration of Fe mg/Kg dry weight basis.

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 [25].

Copper and zinc are mutually competitive as well as competitive to other micronutrients at the carrier sites for plant root uptake [26].

From comparison of both winter, and summer season samples of *Spinacia oleracea* which were not supplemented revealed that 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 was 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.

Experimental

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 into 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 1-12 represent one sample grown in these boxes (twelve samples of one vegetable) which was supplemented either with individual element or combination of elements except in 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 (0-15 cm, 15-30 cm & 30-45 cm) 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, Planting, and Fertilizing Seeds

Spinacia oleracea seeds were planted about 1/2-inch deep in the soil. Two dozen seeds were sown 1-inch apart in three rows with eight seeds per row.

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. The purpose of this

supplementation was to provide the vegetable samples the soil environment with different mineral concentrations, so that their uptake in both the seasons could be evaluated.

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
Sub-boxes:
B-5……. Ind-5. Cr
B-6……. Ind-6. Zn
B-7……. Ind-7. Mg
B-8……. Com-8. Cr, Mg, Zn
Box-C
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 specified volume of these elements was taken (Table-7) in a one liter beaker, added tap water, and this was then spread around 1"-2" area of roots of *Spinacia oleracea* samples grown in different sub boxes except box-C-12.The samples in winter, and summer seasons were supplemented after 10, and 13 days respectively. The beaker was rinsed many times with approximately three liters 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. *Spinacia oleracea* grown in winter was harvested after 44days, and in summer after 35 days.

Watering Plants

In winter *Spinacia oleracea* was watered 14 liters per sub-box once a week, and in summer 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.

Care Throughout 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 vegetable samples 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 $^{\circ}$C until the weight became stable. Samples were then ground with mortar, and 0.5 g of each sample was wet digested with nitric acid (HNO_3): perchlorate ($HClO_4$) (2:1) for 2-3 h on heating mantle [27]. 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, 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 $^{\circ}$C [28]. Texture was determined by Bouyoucos Hydrometer method using Na_2CO_3 as dispersing agent [15] Organic matter by Walky, and Black method as described by Jackson [29] Lime content ($CaCO_3$) by the acid neutralization method according to Black [30].

Table-7: 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	55.85 mg
Zn	6.537	25	163.425 mg
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

Potassium (K) by Flame Photometer, phosphorous (P) by rapid, and sensitive colorimetric method using Uv-visible spectrophoto-meter (Shimadzu, 1700 Japan). DTPA-Extractable minerals in each soil sample were estimated with the help of Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer 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, Aldrich USA. The filtrate was then analyzed for extractable, Cu, Fe, Zn, Cr, Mg, and Mn [31].

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 [32].

Fertilizers and Minerals Salts Applied [33]

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 SO_4. $7H_2O$, and Copper Sulfate (Sigma) Cu $SO_4.5H_2O$.

Conclusion

The present study revealed that season has important effect on plant minerals absorption. By changing the soil minerals environment the uptake of required mineral content of vegetables, perhaps could be enhanced. Thus vegetables can be enriched with selected minerals of nutritional and therapeutic importance through their roots. This will reduce serving size of vegetables, and increase dietary intake of these minerals.

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