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Effect of Applied Zinc and Organic Manures on Iron, Copper and Manganese Contents in Spinach (*Spinacea oleracea* L.)

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Abstract A greenhouse pot experiment was undertaken to study the effect of applied zinc (Zn) on iron (Fe), manganese (Mn) and copper (Cu) content in edible portion of Spinach (*Spinacea oleracea* var All green). Four levels of Zn (0, 5, 20 and 40 mg kg^{-1}) and three levels of organics (control, 3% farmyard manure and 3% poultry manure) were applied to assess the Fe, Mn and Cu content in leaf of Spinach. Results indicated that Iron content in shoot of Palak (first cutting) progressively decreased with the application of zinc up to 40 mg kg^{-1} over control. On an average, plant iron content reduced to the tune of 15.7, 20.48 and 33.09% at 5, 20 and 40 mg Zn kg^{-1}, respectively over control. More or less similar extent of reduction in Mn content in shoot was recorded at different levels of applied Zn. However, effect of Zn application on Cu content was statistically non-significant. On an average, Fe and Mn content in Spinach were found to be increase due to the application of both FYM and poultry manure over control (no organic) and application of poultry manure increased the Cu content in Palak over control.

Keywords Leafy vegetable, Micronutrient, Organic, Zinc fertilization.

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Introduction

Generally the regions in the world with zinc (Zn) deficient soils are also characterized by widespread zinc (Zn) deficiency in humans [1]. Recent estimates indicate that nearly half of the world population suffers from Zn deficiency. Hence, enrichment of edible portion of various crops should constitute a priority of research. Since, cereal crops play an important role in satisfying calorie intake in developing world, a few studies in this line has already been taken up targeting cereal crops. Convincing evidence about the role of Zn fertilization in improving grain Zn concentration in wheat i.e. agronomic bio-fortification has been obtained in field trials of Turkey. Such study needs to be carried out in other crops such as leafy vegetables as well because generally leafy green vegetables are accumulator of metals including Zn. However, along with Zn, other trace metals such as Fe, Mn and Cu are also very important for sustenance of various physiological and metabolic processes in humans [2]. Application of zinc may compete with absorption of other micronutrient cations by plants reducing the micronutrient contents in plants [3, 4] Particularly in such peri-urban agricultural lands, accumulation of substantial amount of Zn in soil has been reported in various studies [5, 6]. Practically, such information on interactive effect of applied Zn and organics on the absorption of other micronutrients by leafy vegetable crops is scanty. Such type of information should prove to be helpful in devising the appropriate agronomic strategy in enriching the micronutrient contents in edible portion of crops. Hence, an attempt has been made to study the iron, copper and manganese content in leafy vegetable as affected by soil applied Zn, FYM and PM. With the above background, the present investigation was undertaken to study the effect of applied zinc, FYM and poultry manure on iron, copper and manganese content in leafy vegetables (*Spinacea oleracea*).

Materials and Methods

Collection and characterization of soil samples and organics

To conduct the pot experiment, bulk surface (0—15 cm) soil was collected from the Agricultural Research Farm of Banaras Hindu University, Varanasi. Soil samples were air-dried, ground and sieved to pass through 2 mm sieve. Soil pH, electrical conductivity (EC), cation exchange capacity (CEC) and organic carbon were determined using standard procedures [7]. For available micronutrient cations, soils were extracted with 0.005 M DTPA—0.01 M $CaCl_2$ [8] and concentration of zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) in the extracts was determined by atomic absorption spectrophotometer (AAS–7000). The soil pH (1:2 soil—water) was 8.20. The values of EC in supernatant liquid of same soil–water suspension were determined to be 0.26 dS m^{-1}. Organic carbon was estimated to be 0.37%. DTPA extractable Zn, Cu, Fe and Mn in soil were 0.78, 2.16, 34.0 and 7.13 mg kg^{-1}, respectively. Texture of soil was sandy clay loam. FYM and poultry manure were collected from Agricultural Research Farm of Banaras Hindu University, Varanasi. FYM and poultry manure were air-dried, ground and sieved to pass through 2 mm sieve. The processed FYM and poultry manure were used for greenhouse experiment. Total organic carbon content in FYM and poultry manure was determined by wet oxidation method [9]. For total metal content, FYM and poultry manure samples were digested in di-acid mixture (HNO_3 : $HClO_4$: : 9 : 4). The di-acid extract was analyzed for Zn, Cu, Fe and Mn contents using Atomic Absorption Spectrophotometer (AAS–7000). Total organic carbon in FYM and poultry manure were 40.9 and 8.13%, respectively. The contents of total Zn, Cu, Fe and Mn in FYM were 154, 62.0, 5200 and 264 mg kg^{-1}, respectively. Total Zn, Cu, Fe and Mn in poultry manure were 305, 34, 1905 and 412 mg kg^{-1}, respectively.

Greenhouse pot experiment and experimental design

A greenhouse pot experiment was conducted to assess study the effect of applied zinc (Zn) on iron (Fe), manganese (Mn) and copper (Cu) content in edible portion of Spinach (*Spinacea oleracea* var All green) grown on soil amended with farmyard manure (FYM) and poultry manure. For the soil, the treatments consist of four levels of Zn, i.e. 0, 5, 20 and 40 mg kg^{-1} soil and three levels of organics, i.e. control, 3% FYM and 3% PM. All twelve treatment combinations (3 organics × 4 levels of Zn) for each crop were replicated thrice and experiments were laid out in completely randomized design. For the greenhouse study, plastic pots of 5 kg capacity were filled with four kg of soil. A uniform basal dose of N : P_2O_5 : K_2O @ 11.1 : 11.1 : 22.2 mg kg^{-1} soil was added in solution form to the soil of each pot through urea, diammonium phosphate and muriate of potash, respectively. Farmyard manure (FYM) and PM @ 3% on weight basis were added in powder form and thoroughly mixed with soil. Zinc was applied as $ZnSO_4.7H_2O$ in solution form. The soil in each pot was then irrigated with tap water and the pots were maintained at field capacity moisture for one month. After one month, seeds of the crop (10–12 Palak 8) were sown and a uniform plant population (Palak : 8) was maintained in each pot after a fortnight of germination. Pots were watered daily with required amount of water on weight basis to

Table 1. Influence of applied Zn on DTPA extractable Zn (mg kg^{-1}) in soils after the harvest of Spinach. FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	DTPA extractable Zn
Zinc levels (Zn) (mg kg^{-1})	
Zn_0	2.14
Zn_5	3.29
Zn_{20}	6.14
Zn_{40}	12.41
SEm±	0.14
CD (p = 0.05)	0.40
Organic manures (OM)	
No organics (Control)	6.10
PM (3%)	5.85
FYM (3%)	6.04
SEm±	0.12
CD (p = 0.05)	0.35
Zn × OM	S

maintain field capacity. First cutting of Palak (above ground edible portion) was taken at 30 days after sowing (DAS). After first cutting, Palal was top-dressed with N @ 11.1 mg kg^{-1} (approx.), and irrigation water. The second cutting of Palak was taken at 55 DAS. Fresh weight of the plant samples was recorded and plant samples were washed with tap water followed by dilute HCl (0.1 N) and finally rinsed with distilled

Table 2. Interactive effect of applied Zn and organics on DTPA extractable zinc (mg kg^{-1}) after the harvest of Spinach. FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	No organics (control)	PM	FYM	Mean
Zn_0	1.8	2.38	2.23	2.14
Zn_5	2.92	3.46	3.24	3.21
Zn_{20}	5.49	5.94	6.98	6.14
Zn_{40}	13.95	11.21	11.38	12.18
Mean	6.04	5.75	5.96	
Zn	SEm±	0.25	CD (p=0.05)	0.52
OM	SEm±	0.22	CD (p=0.05)	0.45
Zn×OM	SEm±	0.44	CD (p=0.05)	0.90

Table 3. Effect of applied Zn and organic manures on iron content (mg kg^{-1}) in Spinach (first cutting and second cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	First cutting (30 DAS)	Second cutting (55 DAS)
Zinc levels (Zn) (mg kg^{-1})		
Zn_0	368.88	348.00
Zn_5	310.55	301.44
Zn_{20}	293.33	291.56
Zn_{40}	275.88	281.78
SEm±	2.28	3.77
CD (p = 0.05)	6.69	11.05
Organic manures (OM)		
No organic (Control)	288.75	272.75
PM (3%)	336.50	333.17
FYM (3%)	311.25	311.17
SEm±	1.97	3.26
CD (p = 0.05)	5.79	9.57
Zn×OM	S	S

water. Plant samples were first air-dried and then oven-dried in hot air oven at 60 ± 5^{0}C until the attainment of constant weight. The dry biomass yield was recorded. Plant samples were ground and digested in a di-acid mixture of HNO_3 / $HClO_4$ (9 : 4) on an electric hot plate and analyzed for Zn content using atomic absorption spectrophotometer. Post-harvest soil was

Table 4. Interactive effect of applied Zn and organic manures iron content (mg kg^{-1}) in Spinach (first cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	No organics (control)	PM	FYM	Mean
Zn_0	344.66	385	377	368.89
Zn_5	293.66	326	312	310.55
Zn_{20}	270	320	290	293.33
Zn_{40}	246.66	315	266	275.89
Mean	288.75	336.50	311.25	
Zn	SEm±	3.23	CD (p=0.05)	6.69
OM	SEm±	2.80	CD (p=0.05)	5.80
Zn×OM	SEm±	5.59	CD (p=0.05)	11.59

Table 5. Interactive effect of applied Zn and organic manures on iron content (mg kg^{-1}) in Spinach (second cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure.

Treatments	No organics (control)	PM	FYM	Mean
Zn_0	305.33	371.67	367	348
Zn_5	287	312.67	304.67	301.45
Zn_{20}	255.33	326.67	292.67	291.56
Zn_{40}	243.33	321.67	280.33	281.78
Mean	272.75	333.17	311.17	
Zn	SEm±	5.33	CD (p=0.05)	11.05
OM	SEm±	4.62	CD (p=0.05)	9.57
Zn×OM	SEm±	9.23	CD (p=0.05)	19.14

taken out of each pot, air-dried, ground and passed through 2 mm sieve and analyzed for pH [7] and DTPA extractable Zn content [8].

Statistical analysis

Analysis of variance method was followed to assess the effect of applied zinc (Zn) on iron (Fe), manganese (Mn) and copper (Cu) content in edible portion of Spinach (*Spinacea oleracea* var All green) adopting factorial concept through completely randomized design [10].

Table 6. Effect of applied Zn and organic manures on copper content (mg kg^{-1}) in Spinach (first cutting and second cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	First cutting (30 DAS)	Second cutting (55 DAS)
Zinc levels (Zn) (mg kg^{-1})		
Zn_0	20.21	24.52
Zn_5	19.75	23.45
Zn_{20}	19.75	22.30
Zn_{40}	19.31	21.35
SEm±	1.33	1.21
CD (p = 0.05)	3.92	3.55
Organic manures (OM)		
No organic (Control)	18.29	28.41
PM (3%)	22.67	20.43
FYM (3%)	18.30	19.86
SEm±	1.15	1.04
CD (p = 0.05)	3.39	3.07
Zn×OM	NS	NS

Table 7. Effect of applied Zn and organic manures on manganese content (mg kg^{-1}) in Spinach (first cutting and second cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	First cutting (30 DAS)	Second cutting (55 DAS)
Zinc levels (Zn) (mg kg^{-1})		
Zn_0	128.48	158.55
Zn_5	104.01	131.16
Zn_{20}	87.93	110.52
Zn_{40}	79.56	89.09
SEm±	2.47	2.45
CD (p = 0.05)	7.24	7.20
Organic manures (OM)		
No organic (Control)	94.35	111.47
PM (3%)	103.74	128.71
FYM (3%)	101.90	126.81
SEm±	2.14	2.12
CD (p = 0.05)	6.27	6.23
Zn×OM	NS	S

Results and Discussion

DTPA extractable zinc

On an average, DTPA extractable Zn increased by 0.53, 1.86 and 4.7 fold over control due to application of Zn @ 5, 20 and 40 mg kg^{-1}, respectively. On an average, application of FYM marginally reduced (6.04 mg kg^{-1}) the DTPA extractable Zn in soil as compared to control (6.10 mg kg^{-1}), whereas poultry manure also reduced the extractable Zn in soil (Table 1). Amendments, like FYM, bio-solids and bio-solid compost may affect the bio-availability of heavy metals in soil due to its high content of organic matter, P and Fe [11]. The reduction in DTPA extractable Zn in FYM treated soil [12]. The interactive effect of applied Zn and organics on the DTPA extractable Zn was statistically significant (Table 2).

Table 8. Interactive effect of applied Zn and organic manures on manganese content (mg kg^{-1}) in Spinach (second cutting). FYM–Farm yard manure ; PM–Poultry manure ; OM–Organic manure ; Zn–Zinc.

Treatments	No organics (control)	PM	FYM	Mean
Zn_0	137.87	169.15	168.62	158.55
Zn_5	119.5	137.89	136.09	131.16
Zn_{20}	100.93	116.5	114.13	110.52
Zn_{40}	87.57	91.32	88.39	89.09
Mean	111.47	128.72	126.81	
Zn	SEm±	3.47	CD (p=0.05)	7.20
OM	SEm±	3.01	CD (p=0.05)	6.24
Zn×OM	SEm±	6.01	CD (p=0.05)	12.47

Iron content in spinach

Iron content in shoot of Palak (first cutting) progressively decreased with the application of zinc up to 40 mg kg^{-1} over control (Table 3). On an average, plant iron content reduced to the tune of 15.7, 20.48 and 33.09% at 5, 20 and 40 mg Zn kg^{-1}, respectively over control. The reduction in plant iron content with applied zinc may be related to inhibitory effect of zinc on absorption and translocation of iron and Fe and Mn content in *Chenopodium* were found to be reduced significantly due to the application of both FYM and sludge over control [13]. Besides, antagonistic interaction between Fe and Zn is supposed to be associated with formation of franklinite ($ZnFe_2O_4$) which depresses the availability of both of the metals in soil. Zinc is also reported to strongly inhibit the reduction of Fe^{3+} to Fe^{2+} which negatively affects uptake and translocation of iron in plants. On an average, iron content in shoot of Palak significantly increased due to application of FYM and poultry manure in plant over control (Table 3). This is obviously related to differential iron content in FYM and PM. The interactive effects of applied zinc and organics on plant Fe content was statistically significant (Table 4). The perusal of data revealed that PM had depressing effect on plant iron content as compared to control, where neither Zn nor organic was applied. With combined application of zinc and PM, plant Fe content showed a significant increase at all levels of applied Zn as compared to only zinc treated pots. By and large similar effect of applied Zn and organics on iron content in shoot of Palak in second cutting was obtained as that of first cutting (Table 3, Table 5).

Copper content in spinach

On an average, effect of application of zinc up to 40 mg kg^{-1} on Cu content in shoot of Palak (first cutting) was statistically at par with control (Table 6). On an average, application of poultry manure increased the Cu content in Palak over control and FYM treated soil (Table 6). The two way interaction of applied zinc and organics on Cu content of Palak was statistically non-significant.

Manganese content in spinach

Manganese content in Palak (first cutting) was reduced by 19.04, 31.75 and 37.63% due to application of zinc @ 5, 20 and 40 mg kg^{-1}, respectively (Table 7). Depressing effect of applied zinc on Mn content in plant may be related to antagonistic interaction between these two cations during the absorption by plant roots. On an average, both PM and FYM were found to increase plant Mn content significantly with reference to control (Table 7). However, both of these organic materials enhanced the Mn content significantly in plant over control. Two factor interactions were statistically non-significant in first cutting. Interaction in second cutting is significant as given in Table 8.

Conclusion

It is clear from this study that higher level of applied Zn in soil had strong negative effect on the content of other micronutrient cations like Fe, Mn and Cu. One should be careful about the reduction of other micronutrient cations, particularly Fe in leafy green vegetables while enriching the same with Zn. This will have far reaching practical implications as leafy green vegetables are considered as very good source of Fe also.

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