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HAZARD QUOTIENT FOR INTAKE OF IRON, MANGANESE AND COPPER THROUGH CONSUMPTION OF SPINACH AS INFLUENCED BY APPLIED ZN AND ORGANICS

Seema, Neelam Yadav and Sunita Choudhary¹

Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-2210 05, India, E-mail: seemachahar94@gmail.com and ¹Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi- 2210 05, India, Corresponding Author: Seema

Abstract: Zinc (Zn), Iron, Manganese and Copper is one of the most important micronutrients for humans. Human health is adversely affected by its deficiency. At the same time, excessive intake of these cationic micronutrient may cause several physiological diseases and dis-orders. In this context, risk to human health for intake of Fe, Mn, Cu and Zn-enriched Palak (*Spinacea oleracea* L.) was assessed in terms of hazard quotient (HQ). In a pot culture experiment, spinach crop was grown on soil treated with 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). Results indicated that The values of HQ for intake of Fe through consumption of Palak (first cutting) a mean value of 0.24. The corresponding values for second cutting of Palak a mean 0.23. The values of HQ for intake of Mn a mean value of 0.16 in first cutting of Palak. In second cutting, an average value of 0.20. The concentration of Cu in Palak ranged from 16.44 to 23.66 (mean value 19.76 mg kg⁻¹) and 17.7 to 29.13 mg kg⁻¹ (mean value 22.9 mg kg⁻¹) for first and second cutting, respectively. By and large, this enriched vegetable was safe to be consumed by human as far as their Fe, Mn and Cu contents were concerned.

Keywords: Zinc, Iron, Manganese, Copper, Spinach, Hazard quotient, Organics.

Introduction: Micronutrients, also known as trace elements or trace minerals, include those nutrients are required in extremely small quantities by crops, livestock and humans. This, however, in no way refers to their minor role. These include iron (Fe), copper (Cu), manganese (Mn), zinc (Zn). Deficiencies of these nutrients in crops and livestock may cause serious crop production or animal health problems. Excessive intake of Zn is associated with several physiological diseases and dis-orders in human beings such as lowered activity of a Cu containing enzyme (Cu-Zn-Superoxide dismutase), Fe deficiency and lower levels of high density lipoprotein (HDL) cholesterol^[1]. Trace metals such as Fe, Mn and Cu are very important for sustenance of various physiological and metabolic processes in humans^[2]. Zinc (Zn) is one of the most important essential trace elements in human nutrition with a recommended daily allowances ranging from 5 mg for infants to 15 mg for adults^[3]. It is

essential for the functioning of large number of enzymes which are important (i) during pregnancy (Pregnant women require much more Zn than other wise), (ii) for brain growth of infants, (iii) in immune compliance, and (iv) in playing possible role as anti-carcinogen^[4]. Its abnormal metabolism is related to certain disorders such as diabetic complications^[5]. Oxidative stress has been considered as the major causative factor for diabetic cardiomyopathy. Acute toxicity of this element leads to vomiting, diarrhea and neurological damage in human^[1]. The negative effects of chronic toxicity include lowered activity of Cu-containing enzyme (Cu-Zn-superoxide dismutase), Fe deficiency and lowered levels of high density lipoprotein (HDL) cholesterol. Hence, it is obvious that assessing the suitability of Zn-enriched leafy vegetables for human consumption is equally important as enrichment itself. This is more relevant when one goes for enriching metal accumulator plants like *Palak*. Usually, risk to human health for intake of

metals through consumption of food crops grown on contaminated soils is expressed in terms of hazard quotient ^[6-9]. Fe is also an important element in all living organisms, acting as a key component of haemoglobin and a number of enzymes ^[4]. In enrichment study, it would be worthwhile working out the maximum permissible dose of applied Zn based on hazard quotient (HQ) for dietary intake of Zn through consumption of leafy vegetable. With the above background, the present investigation was undertaken to assess the hazard quotient (HQ) for intake of Fe, Cu, Mn by human through consumption of Spinach (*Spinacea oleracea* L.) grown on soil treated with Zn and organics.

Materials and Methods

Greenhouse Pot Experiment and Experimental Design: A greenhouse pot experiment was conducted to assess the effect of applied zinc, FYM and poultry manure on Zn content in leaves of *Palak* (var. All green) grown on Inceptisol of Varanasi. For 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 ^[10]. For available micronutrient cations, soils were extracted with 0.005 M DTPA—0.01 M CaCl₂ (Lindsay WL, Norvell WA 1978) and concentration of zinc (Zn) 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 in soil were 0.78 mg kg⁻¹. 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 ^[11]. For total metal content, FYM and poultry manure samples were digested in di-acid mixture (HNO₃: HClO₄ : 9:4). The di-acid extract was analysed 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 in FYM and poultry manure were 154 and 305 mg kg⁻¹, respectively. 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: P₂O₅: K₂O @ 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 ZnSO₄.7H₂O 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*) were sown and a uniform plant population (*Palak*: 8) was maintained in each pot after a fortnight of germination. Irrigation was done as per requirement of the water in pots. First cutting of *Palak* (above ground edible portion) was taken at 30 days after sowing (DAS). After first cutting, *Palak* was top-dressed with N @ 11.1 mg kg⁻¹ (approx.), and irrigation water. The second cutting of *Palak* was taken at 55 DAS.

Analysis of Plant Samples: After harvest of *Palak*, fresh weight of the plant samples was recorded and plants were washed with tap water followed by dilute HCl (0.1 N) and finally rinsed with distilled water. Plant samples were dried in hot air oven at 60±5°C. After attaining constant weight, biomass yield was recorded. Dried plant samples were ground and used for subsequent chemical analysis. Plant samples were digested with di-acid mixture (HNO₃:HClO₄:: 9:4) and Zn in the extracts were determined using flame atomic absorption spectrophotometer (FAAS).

Human Health Risk Assessment: The risk to human health (expressed as hazard quotient) for intake of Zn through consumption of *Palak* grown on soil with FYM and PM at different levels of applied Zn was calculated using USEPA Protocol, ^[12]. Following relationship ^[13] was used to calculate the hazard quotient (HQ):

$$HQ_{gv} = (ADD/RfD)$$

Where, HQ_{gv} is the hazard quotient to a human for consumption of green vegetable

(Palak in present investigation); ADD: the average daily dose (mg metal/kg body weight/day) and RfD: reference dose or maximum allowable intake (mg/kg body

weight/day). The values of RfD for Zn were considered as 0.3^[12]. The average daily dose (ADD) was computed using following relationship:

$$ADD = \frac{mc \times cf \times di}{bw}$$

Where, ADD = Where, mc is the metal concentrations in plant (mg kg⁻¹) on dry weight basis, cf is the fresh to dry weight conversion factor for plant samples (calculated as the ratio of dry weight to fresh weight), di is the daily intake of green vegetable (kg) and bw is the body weight (kg) of human. Daily intake of green vegetable was considered as 200 g/person/day which is recommended amount from nutritional point of view^[14]. The conversion factors of 0.08 as obtained in this study were used for converting fresh weight of Palak to dry weight, respectively. Average body weight for an adult was considered as 70 kg. Assessment of risk as computed here is not complete since, metal accumulation to soil organisms, groundwater, surface water, direct uptake of soil by human and animal are some of the other risks which have not been considered here.

Statistical Analysis: Analysis of variance method was followed to assess the effect of applied zinc, FYM and poultry manure on Fe, Cu and Mn content adopting factorial concept through completely randomized design^[15].

Results and Discussion

Fe, Mn and Cu were not applied through fertilizer, a substantial amount of these micronutrients were added through FYM and

poultry manure. The intake of these micronutrients at toxic levels by human beings results in several physiological and metabolic dis-orders^[1]. Excessive intake of Cu leads to Wilson’s disease and Cirrhosis, haemolysis, hepatic necrosis, renal damage and swelling of salivary gland in human. Psychiatric and neurological dis-orders are associated with intake of Mn at toxic level. The toxic effect of ingesting high levels of Fe results in the haemochromatosis and cirrhosis of liver. Besides, metabolic acidosis and shock are also associated with the toxicity of this element. Hence, HQ for intake of these micronutrients through consumption of Palak are computed and presented in tables 1, 2 and 3.

Results indicate that across the treatments Fe content ranged from 246.66 to 385 (312.16 mg kg⁻¹) and 243.33 to 371.67 (305.69 mg kg⁻¹) in shoot of Palak (first cutting, second cutting) (Table 1). The values of HQ for intake of Fe through consumption of Palak (first cutting) varied from 0.19 to 0.29 with a mean value of 0.24. The corresponding values for second cutting of Palak ranged from 0.19 to 0.28 (mean 0.23). Values of HQ for the crop did not rich even up to 0.5. Hence, the Palak is safe to be consumed by human beings as far as their Fe content is concerned.

Table-1: Hazard quotient (HQ) for intake of Iron (Fe) through consumption of Spinach as influenced by applied Zn and organics

Treatment combinations		First cutting (30 DAS)		Second cutting (55 DAS)	
		Fe conc. (mg kg ⁻¹)	HQ	Fe conc. (mg kg ⁻¹)	HQ
Control	0	344.67	0.26	305.33	0.23
	5	293.67	0.22	287.00	0.22
	20	270.00	0.21	255.33	0.19
	40	246.67	0.19	243.33	0.19
PM	0	385.00	0.29	371.67	0.28
	5	326.00	0.25	312.67	0.24
	20	320.00	0.24	326.67	0.25
	40	315.00	0.24	321.67	0.25
FYM	0	377.00	0.29	367.00	0.28
	5	312.00	0.24	304.67	0.23
	20	290.00	0.22	292.67	0.22
	40	266.00	0.20	280.33	0.21
Mean		312.17	0.24	305.69	0.23
Minimum		246.67	0.19	243.33	0.19
Maximum		385.00	0.29	371.67	0.28

Mn concentration in Palak varied from 68.13 to 130.93 and 87.5 to 169.1 mg kg⁻¹ in first

and second cutting, respectively (Table 2). The corresponding mean values were 100 and 122.3

mg kg⁻¹. The values of HQ for intake of Mn varied from 0.11 to 0.21 with a mean value of 0.16 in first cutting of Palak. In second cutting, it varied from 0.14 to 0.28 with an average value of

0.20. So, based on these values of HQ, it can be reduced that intake of this vegetable is not likely to induce Mn toxicity in human.

Table 4.11 Hazard quotient (HQ) for intake of Manganese (Mn) through consumption of Spinach as influenced by applied Zn and organics

Treatment combinations		First cutting (30 DAS)		Second cutting (55 DAS)	
		Mn conc. (mg kg ⁻¹)	HQ	Mn conc. (mg kg ⁻¹)	HQ
Control	0	124.67	0.20	137.87	0.23
	5	100.23	0.16	119.51	0.20
	20	84.38	0.14	100.93	0.16
	40	68.13	0.11	87.57	0.14
PM	0	130.93	0.21	169.16	0.28
	5	106.37	0.17	137.89	0.23
	20	91.09	0.15	116.50	0.19
	40	86.58	0.14	91.32	0.15
FYM	0	129.85	0.21	168.62	0.28
	5	105.46	0.17	136.09	0.22
	20	88.31	0.14	114.14	0.19
	40	83.99	0.14	88.39	0.14
Mean		100.00	0.16	122.33	0.20
Minimum		68.13	0.11	87.57	0.14
Maximum		130.93	0.21	169.16	0.28

The concentration of Cu in Palak ranged from 16.44 to 23.66 (mean value 19.76 mg kg⁻¹) and 17.7 to 29.13 mg kg⁻¹ (mean value 22.9 mgkg⁻¹) for first and second cutting, respectively (Table 3). The maximum value of HQ for intake

of Cu was same (0.01) for first and second cutting of Palak (Table 3). Since the values of HQ for intake of Cu through consumption of Palak are far below 0.5, this vegetable is safe to be consumed by human beings.

Table 4.12 Hazard quotient (HQ) for intake of Copper (Cu) through consumption of Spinach as influenced by applied Zn and organics

Treatment combinations		First cutting (30 DAS)		Second cutting (55 DAS)	
		Cu conc. (mg kg ⁻¹)	HQ	Zn conc. (mg kg ⁻¹)	HQ
Control	0	21.52	0.01	28.68	0.01
	5	16.44	0.01	29.13	0.01
	20	17.44	0.01	28.34	0.01
	40	17.77	0.01	27.51	0.01
PM	0	21.72	0.01	22.52	0.01
	5	23.66	0.01	20.85	0.01
	20	22.99	0.01	19.52	0.01
	40	22.33	0.01	18.85	0.01
FYM	0	17.40	0.01	22.36	0.01
	5	19.16	0.01	20.36	0.01
	20	18.83	0.01	19.03	0.01
	40	17.83	0.01	17.70	0.01
Mean		19.76	0.01	22.90	0.01
Minimum		16.44	0.01	17.70	0.01
Maximum		23.66	0.01	29.13	0.01

Conclusion: It can be concluded from the study that there is ample scope of enriching *Palak*. The level of applied Zn may go up to 40 mg kg⁻¹. Fe, Mn and Cu were not applied through fertilizer, a substantial amount of these micronutrients were added through FYM and poultry manure. Even application of Poultry manure did not enrich of this vegetable with Fe, Cu, Mn to toxic levels. The values of HQ for intake of Fe, Mn, Cu through consumption of Palak are far below the critical limit, Spinach is safe to be consumed by human beings.

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