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ZINC INDEXING IN WHEAT GRAINS AND ASSOCIATED SOILS OF SOUTHERN PUNJAB

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Higher grain Zn concentration is desirable to combat human Zn deficiency. A total of 58 farmers' fields were surveyed from districts of Multan and Lodhran to represent cotton-wheat rotation zone of Punjab. Soils were sampled separately from the surface (0–15 cm depth) and subsurface (15–30 cm depth) layers. Within 5 m^2 of soil sample, about 100 spikes from mature tillers of wheat were also collected. Nearly all subsurface and 76% of the collected surface soil samples were deficient in plant available Zn resulting in grain Zn concentration of 20 $\mu g\ g^{-1}$, on average. The inherent capacity of subsurface soil layer to supply Zn positively correlated with grain Zn concentration (r=0.33, n=58; P=0.01). Organic matter content (r=0.32, n=58; P=0.02) in subsurface layer and soil salinity (EC_e) of both layers (r=−0.53, n=58; P<0.01 for EC_e of both layers) were the other major soil characteristics that significantly correlated with grain Zn concentration. Zinc bioavailability in wheat grains, estimated by [phytate]:[Zn] ratios and trivariate model of Zn absorption, was low. Present study indicated a need to reduce current grain [phytate]:[Zn] ratio (46, on average) and to increase the current trivariate model based estimated Zn bioavailability (1.5 mg Zn for 300 g flour, on average) by about 2-folds. Conclusively, Zn fertilization strategy for southern Punjab was suggested as a solution to human Zn deficiency and low grain yields.

Keywords: Biofortification; Calcareous soils; Southern Punjab; Wheat; Zinc

INTRODUCTION

More than 77% of total wheat production in Pakistan is contributed by Punjab province (Bureau of Statistics, 2009). The Punjab province is divided into rain-fed and irrigated regions, with the irrigated region producing most of the wheat. The irrigated region is further divided into various zones on the basis of general crop rotation. In contrast to the people living in rice-wheat rotation zone, wheat bread is the sole staple food in the cotton-wheat rotation zone (most of the southern Punjab). Therefore, wheat grains are the major source of minerals, including Zn, and calorie (> 85%) intake for the people living in villages in the cotton-wheat rotation zone.

Cereals, wheat and rice in particular, suffer from Zn deficiency (Bell and Dell, 2008). In contrast to rice-wheat rotation zone of Pakistan, where Zn application is normally practiced to rice crop, Zn is not applied to soils in the cotton-wheat rotation zone. Moreover, Zn studies in the past were focused more on the rice-wheat rotation zone due to the appearance of Zn deficiency in rice crop and a significant increase in paddy yield with Zn application to flooded soil conditions (Alloway, 2008; Imtiaz *et al.,* 2010; Rehman *et al.,* 2012). The cotton-wheat rotation zone has received insignificant consideration for Zn indexing and application studies. As a result, there is lack of up to date information regarding the status of Zn in this zone.

Cereal grains produced on Zn-deficient soils of India, Pakistan, China, Iran and Turkey are the main reason of severe Zn deficiency problem in human population of these countries (Alloway, 2009; Hotz and Brown, 2004). Similar to most other soils in Pakistan, the soils in the cotton-wheat rotation zone are alkaline and calcareous in nature and are located in arid to semiarid zone. Such soil conditions contribute to Zn deficiency (Alloway, 2008; Ahmad *et al.,* 2012) which is the third most widespread nutritional constraint to crop production in Pakistan (Hamid and Ahmad, 2001). Moreover, Zn is essentially required by humans and Zn deficiency in soils correlates with human Zn deficiency (Abrahams, 2002; Alloway, 2009). In Pakistan, about 40% children and women are facing Zn deficiency (Ministry of Health, 2009). The extant is more in rural areas due to low Zn in their staple diet that is wheat. On an average, about

90% human population in Pakistan is under the risk of Zn deficiency.

Due to economic reasons, industrial fortification and clinical supplementation are not viable strategies to combat human Zn deficiency in developing countries at large scale. Biofortification of wheat grain produced on calcareous soils of the cotton-wheat rotation zone is required to reduce the risk of human Zn deficiency in the area. Before selecting a biofortification strategy, nutrient indexing of Zn in wheat grains and associated alkaline and calcareous soils of the zone is prerequisite to determine the current levels of Zn in wheat grains and associated soils. In the present study, 58 farmers' fields located in cotton-wheat rotation zone of Punjab were surveyed for wheat grain and soil samples. The main objectives of the study were to: i) estimate the extent of soil Zn deficiency in cotton-wheat rotation zone of Punjab (southern Punjab); ii) determine concentration of Zn, concentration of phytate and Zn bioavailability in grains of wheat grown at formers' fields; and iii) study the relationship of soil physicochemical properties with grain Zn concentration.

MATERIALS AND METHODS

Site selection: Multan and Lodhran districts from Southern Punjab were taken as representative of major cotton-wheat rotation zone. The climate is arid to semi-arid and annual rainfall is about 185 mm that is mostly during monsoon season.

Soil and grain sampling: A total of 58 farmers' fields from the cotton-wheat rotation zone (districts of Multan and Lodhran) of Punjab (Pakistan) were randomly selected. Soil from the selected fields was sampled separately from the surface (0–15 cm depth) and subsurface (15–30 cm depth) layers. Within 5 m^2 of soil sample, about 100 spikes from mature tillers of wheat were also collected. The soil and plant samples, collected in triplicates, were properly labeled and transported to the laboratory for analysis (James and Wells, 1990; Munson and Nelson, 1990). Soil samples were dried and ground to pass through a 2-mm sieve. A portion of the sieved soil samples was analyzed for various soil properties (Table 1).

Grain Zn and phytate concentration: Collected spikes were manually threshed to separate grains. Grain samples were washed briefly with distilled water and rapidly dried with tissue papers before drying in a forced-air-driven oven at 65°C for 48 h. The dried samples were ground in a mill to pass through a 0.5-mm sieve. A subsample of plant material was digested in a di-acid mixture (HNO_3:$HClO_4$ ratio of 2:1) (Jones and Case, 1990). Zinc concentration in the digest was measured by an atomic absorption spectrophotometer (PerkinElmer, AAnalyst 100, Waltham, USA). For phytate determination, 60 mg sample of finely ground grains was extracted with 10 mL of 0.2 N HCl at room temperature for 2 h under continuous shaking. Phytate in the extract was determined by an indirect method (Haug and Lantzsch, 1983) on a spectrophotometer (Shimadzu, UV-1201, Kyoto, Japan). All grain samples for mineral and phytate determinations were prepared and analyzed in duplicates.

Zinc bioavailability: Zinc bioavailability was qualitatively estimated as [phytate]:[Zn] ratio in grains (Brown *et al.,* 2001; Weaver and Kannan, 2002) and quantitatively estimated by employing trivariate model of Zn absorption (Hambidge *et al.,* 2010; Miller *et al.,* 2007). The trivariate model estimates total daily absorbed Zn (TAZ) (mg Zn d^{-1}) by implying total daily dietary phytate (TDP) (mmol phytate d^{-1}) and total daily dietary Zn (TDZ) (mmol Zn d^{-1}):

$$\mathrm{TAZ} = 0.5 \cdot \left(0.091 + \mathrm{TDZ} + 0.680 \cdot \left(1 + \frac{\mathrm{TDP}}{0.033}\right) - \sqrt{\left(0.091 + \mathrm{TDZ} + 0.680 \cdot \left(1 + \frac{\mathrm{TDP}}{0.033}\right)\right)^2 - 0.364 + \mathrm{TDZ}} \right)$$

The TAZ was estimated for 300 g of wheat flour, which is roughly equivalent to per capita consumption in Pakistan (FAO, 2012), and was termed as estimated Zn bioavailability (Rosado *et al.,* 2009).

Statistical analysis and map construction: Pearson correlations coefficients and other statistical computations were made (Steel *et al.,* 1997) by using a PC based software, *Statistix 9® for Windows* (Analytical Software, Tallahassee, USA). The software used for the geochemical mapping was ARCMAP version 10. The Inverse Distanced Weighted

Table 1. Physicochemical properties of surface (15–30 cm depth) soil samples collected from 58 farmers' fields

Soil characteristic		Unit	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Mean±SD
[1]Particle size distribution	**sand**	%	8	18	24	30	55	25±10
	silt		15	42	47	50	62	46±8
	clay		7	25	31	34	40	30±6
[2]$CaCO_3$		g kg^{-1}	23	49	62	78	103	64±19
[3]EC_e		dS m^{-1}	1	2	3	5	17	4±4
[4]pH_s		---	7.4	7.6	7.7	7.8	8.2	7.7±0.2
[5]Organic matter		g kg^{-1}	3	7	9	11	17	9±3
[6]Plant available Zn		mg kg^{-1}	0.1	0.3	0.4	0.7	1.2	0.5±0.3

[1]Hydrometer method (Gee and Bauder, 1986); [2]Acid dissolution (Allison and Moodie, 1965); [3]Electric conductivity of saturated soil paste extract; [4]pH of saturated soil paste; [5]Walkley-Black method (Nelson and Sommers, 1982); [6]Extraction with 0.005 M DTPA (Lindsay and Norvell, 1978)

method was used for the interpolation of geographical data.

RESULTS

Soil Properties and Extent of Soil Zn Deficiency: The surveyed fields were alkaline with pH_s ranging from 7.4 to 8.2 for the surface layer (Table 1) and from 7.4 to 8.5 for the subsurface soil layer (Table 2). Calcium carbonate content, a measure of calcareousness of soil, ranged from 23 to 103 g kg^{-1} and 13 to 108 g kg^{-1}, respectively for the surface and subsurface soil layers. About 35% of the sampled fields were saline ($EC_e > 4$ dS m^{-1}) indicating salinity hazard in the zone. Moreover, half of surface and subsurface soil samples had $EC_e > 3$ dS m^{-1}. On average, organic matter in the soil samples was less than 9 g kg^{-1} in the surface layer while maximum organic matter content was 10 g kg^{-1} in the subsurface layer. Particle size analysis indicated that silt was the major soil fraction (about 45% on average) found in the zone. Clay loam and silty clay loam were major soil textural classes in the zone.

Average plant available Zn, extracted by 0.005 M DTPA, was 0.5 in the surface soil layer (Table 1) while 0.3 in the subsurface soil layer (Table 2). More then 75% of surface soil samples had less than 0.7 mg Zn kg^{-1} soil while the same percentage of subsurface soil samples was lower than 0.4 mg Zn kg^{-1} soil fig.1. Zinc concentration ranged from 0.09 to 1.21 mg kg^{-1} in the surface soil and from 0.03 to 0.9 mg kg^{-1} in the subsurface soil. Based on a critical level of 0.75 mg kg^{-1} (Bansal *et al.,* 1990), 19, 40, 17% of the surface soil samples have severe, medium and marginal Zn deficiency problem, respectively (Fig. 1). Nearly all subsurface soil samples were deficient in plant available Zn. Developed maps of soil Zn status by inverse distance weighting indicating that major area was sever deficient in

Table 2. Physicochemical properties of subsurface (15–30 cm depth) soil samples collected from 58 farmers' fields

Soil characteristic		Unit	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Mean±SD
[1]Particle size	**sand**		12	18	25	30	55	26±10
distribution	**silt**	%	23	41	47	50	62	45±8
	clay		17	24	29	34	40	29±6
[2]$CaCO_3$		g kg^{-1}	13	53	68	78	108	65±20
[3]EC_e		dS m^{-1}	1	2	3	6	14	4±3
[4]pH_s		---	7.4	7.6	7.8	8.0	8.5	7.8±0.2
[5]Organic matter		g kg^{-1}	0	4	6	7	10	6±2
[6]Plant available Zn		mg kg^{-1}	0.0	0.1	0.2	0.4	0.9	0.3±0.2

[1]Hydrometer method (Gee and Bauder, 1986); [2]Acid dissolution (Allison and Moodie, 1965); [3]Electric conductivity of saturated soil paste extract; [4]pH of saturated soil paste; [5]Walkley-Black method (Nelson and Sommers, 1982); [6]Extraction with 0.005 M DTPA (Lindsay and Norvell, 1978)

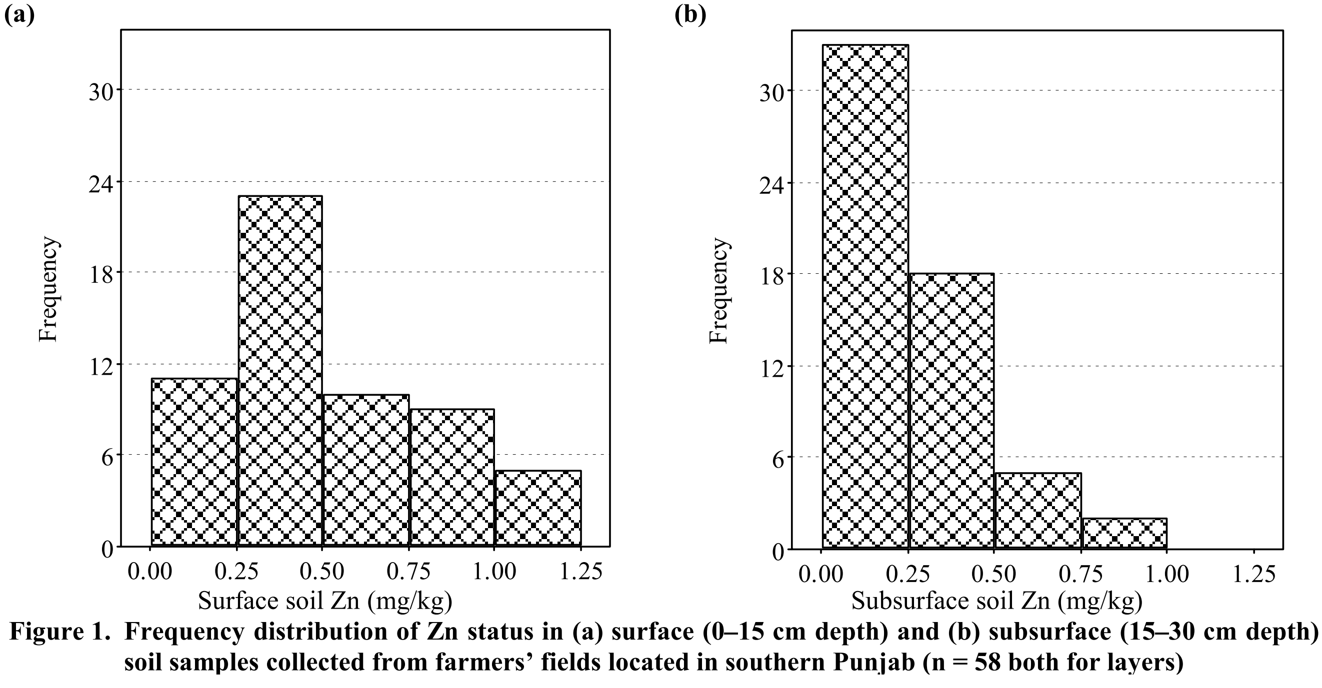

Figure 1. Frequency distribution of Zn status in (a) surface (0–15 cm depth) and (b) subsurface (15–30 cm depth) soil samples collected from farmers' fields located in southern Punjab (n = 58 both for layers)

phytoavailability of Zn from subsurface layer and medium deficient in phytoavailability of Zn from surface layer (Fig. 2).

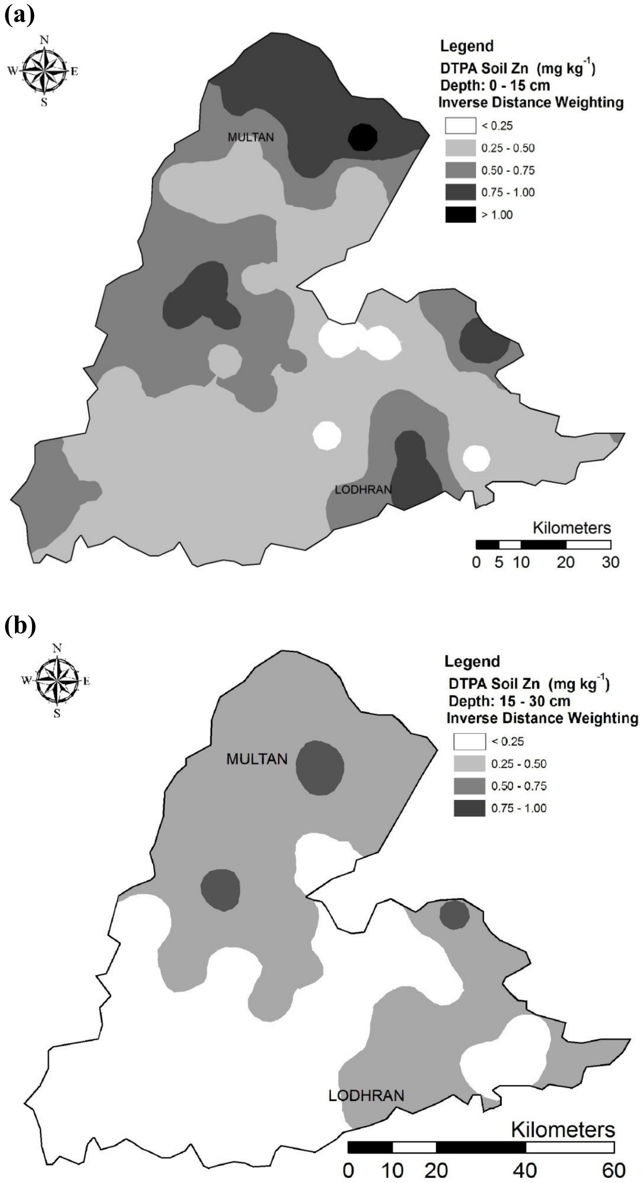

Figure 2. Extent of Zn deficiency in (a) surface and (b) subsurface soil layers of soils in southern Punjab (n = 58 for both layers)

Grain Zn and its Bioavailabilit: Grain Zn concentration in the samples, collected from 58 fields in cotton-wheat rotation zone of Punjab (Pakistan), ranged from 11 to 35 µg g^{-1} with most of the samples having 20±4 µg Zn g^{-1} (Table 3). Phytate concentration also varied in the collected grain samples. The average value of phytate concentration was 10 mg g^{-1} and ranged from 6 to 14 mg g^{-1}.

Grain phytate concentration, together with grain Zn concentration, translated into an average [phytate]:[Zn] ratio of 46 (table 3). Due to wide variation in grain phytate and Zn concentration among the fields, however, [phytate]:[Zn] ratio ranged from 19 to 89. Estimated Zn bioavailability in wheat grains ranged from 0.8 to 2.4 mg Zn for 300 g flour. Average estimated Zn bioavailability was 1.5 mg Zn for 300 g flour and about 75% of the sampled grains had lower than the average estimated Zn bioavailability.

Relationship among soil Zn status, other soil properties and grain Zn concentration: The DTPA-extractable Zn in the soil layers had a negative relationship with soil EC_e ($r = 0.25$, $P = 0.05$ for subsurface soil layer; $n = 58$) and strong positive relationship with soil organic matter ($r = 0.37$ or higher, $n = 58$, $P < 0.01$ for both layer) (Table 4). Grain Zn concentration was correlated with various properties of the surface and subsurface soil layers. In contrast to plant available Zn in the surface layer, Zn in subsurface soil layer had a significant positive relationship ($r = 0.33$, $n = 58$; $P = 0.01$) with grain Zn concentration. Organic matter in subsurface ($r = 0.32$, $n = 58$; $P = 0.02$) layer also had a significant positive relationship with grain Zn concentration. However, the EC_e of subsurface layer had a negative relationship ($r = -0.53$, $n = 58$; $P<0.01$) with grain Zn concentration.

DISCUSSION

The cotton-wheat rotation zone is the most productive zone of Pakistan. The soils of the zone are characterized, as confirmed in the present survey (Table 1 and 2), by high pH, high $CaCO_3$ content and low organic matter content (Rafiq, 2005). Soil analysis indicated that 35% of the surveyed fields were saline. This could be due to application of underground water for irrigation which contained substantial ($EC > 1$ dS m^{-1}) amounts of salts (Ghafoor *et al.,* 2004). Arid climate and parent materials are the other causes for salinity in the region.

Table 3. Zinc concentration, phytate concentration and Zn bioavailability estimates in wheat grains collected from 58 farmers' fields

Grain composition	Unit	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Mean±SD
Zn	µg g^{-1}	11	17	20	23	35	20±5
Phytate	mg g^{-1}	6	9	10	11	14	10±2
[Phytate]:[Zn] ratio	---	19	40	44	50	89	46±12
Estimated Zn Bioavailability	mg for 300 g	0.8	1.3	1.4	1.5	2.4	1.5±0.3

Table 4. Relationship of Zn in soils (DTPA-extractable Zn) and wheat grains with various physicochemical characteristics of soil sample collected from 58 farmers' fields

Parameters	$CaCO_3$	EC_s	Organic matter	pH_s	Sand	Silt	Clay	Soil Zn
Surface soil samples (0–15 cm depth)								
Soil Zn	−0.16	−0.23	0.37	−0.04	−0.21	0.19	0.08	1.00
***P* value**	0.23	0.08	<0.01	0.78	0.11	0.14	0.55	0.00
Grain Zn	−0.24	−0.53	0.19	−0.23	−0.04	0.03	0.03	0.16
	0.06	<0.01	0.16	0.08	0.76	0.84	0.82	0.24
Subsurface soil samples (15–30 cm depth)								
Soil Zn	−0.16	−0.25	0.39	0.16	−0.01	−0.03	0.05	1.00
	0.23	0.05	<0.01	0.24	0.95	0.85	0.71	0.00
Grain Zn	−0.18	−0.53	0.32	−0.17	0.13	−0.06	−0.14	0.33
	0.19	<0.01	0.02	0.20	0.32	0.67	0.28	0.01

n = 58 for both surface and subsurface soil layers

About 75% of surface soil samples were deficient in plant available Zn and about 65% of the remaining fields were only marginally optimum in plant available Zn (Fig. 1). The widespread Zn deficiency in the zone (Fig. 2) can be attributed to high $CaCO_3$, high pH and low organic matter (Alloway, 2009). As surface soil layer is under direct influence of agricultural practices, soil organic matter and DTPA-extractable Zn were more in the surface layer as compared to subsurface layer. Moreover, both DTPA-extractable Zn and organic matter had a significant ($r = 0.32$, $n = 58$; $P = 0.02$ for subsurface soil layer) positive relationship (Table 4) indicating a positive role of organic matter in increasing the availability of Zn (Marschner and Rengel, 2012). Due to high Zn retention capacity of calcareous soils, calcareousness of soil (high $CaCO_3$) had a negative relationship with plant available Zn in the soil layers (Hussain *et al.,* 2011). However, the relationship was weak in present study, particularly due to several confounding factors.

There were wide variations in grain Zn concentration among the fields surveyed in this study (Table 3). Zinc concentration ranged from 11 to 35 µg g^{-1} in grains collected from various locations. The fields for plant and soil sampling were randomly selected without consideration of the wheat varieties. Therefore, this variation in grain Zn concentration was partially due genotypic differences in the collected wheat grain samples (Murphy *et al.,* 2008; Nadim *et al.,* 2011). However, the inherent capacity of soil to supply Zn (DTPA-extractable Zn) determined grain Zn concentration (Table 4). As reported in a previous investigation (Rafique *et al.,* 2006), the relationship was much stronger ($r = 0.33$, $n = 58$; $P = 0.01$) between grain Zn concentration and subsurface soil Zn. Moreover, other soil properties also influenced plant Zn status by determining the availability of Zn from soil to plant roots (Marschner and Rengel, 2012). In the present study, salt concentration in soil solution was found to greatly reduce grain Zn concentration. This could be due to competition of cations for uptake by plant roots and reduced plant growth. Similarly, subsurface organic matter had a positive relationship ($r = 0.32$, $n = 58$; $P = 0.02$) with grain Zn concentration. Therefore, the study confirmed the importance of subsurface soil layer in better Zn nutrition of wheat plants.

Average grain Zn concentration from the fields sampled in this study was 20 µg g^{-1}. This indicated that about 2.5 times increase in grain Zn concentration is required for better human nutrition (Cakmak, 2008). Moreover, grains of Pakistani wheat varieties had lower than desired levels of Zn concentration (Hussain *et al.,* 2012a,b). Therefore, wheat grains should be biofortified by either application of Zn fertilizer to wheat crop or breeding for higher grain Zn concentration (Rengel *et al.,* 1999; Velu *et al.,* 2011). Due to widespread soil Zn deficiency, which is resulting in lower yields and grain Zn concentration, application of Zn fertilizers to soils seems more justifiable (Cakmak, 2009). Zinc fertilization, under such situations, is a rapid solution to human Zn deficiency and a complementary approach to breeding for biofortification programs (Cakmak, 2008).

Phytate concentrations in food hinders Zn absorption into human body by making stable complexes with Zn (Turnlund *et al.,* 1984). Collected grain samples significantly varied in grain phytate concentration (Table 3). However, range of grain phytate concentrations was similar as reported by Lott et al. (2000) in a global estimate. Variation in grain phytate concentrations is partially due to genotypic variations in wheat varieties and differential soil properties. However, these were probably greatly influences by the amount of P fertilizer applied to wheat crop (Ryan *et al.,* 2008). As P application is required for optimum yield of wheat crop, one cannot reduce rate of P application to soil.

Dietary Zn requirement of an adult is ranges from 10 to 13 mg per day, if bioavailability of food is medium (Brown *et al.,* 2001). However, bioavailability of Zn to humans is explained both by the amounts of dietary Zn and dietary phytate. Physiological requirement is about 3 mg per day that can be achieved by intervening more Zn and less phytate (Institute of Medicine, 2001). The wheat grains sampled for the study had average estimated Zn bioavailability of 1.5 mg Zn for 300 g flour and [phytate]:[Zn] ratio of 46 (Table 3). However, desired levels are 3 mg estimated Zn bioavailability in 300 g flour and [phytate]:[Zn] ratio of less than 20 (Institute of Medicine, 2001; Turnlund *et al.,* 1984).

Zinc application increases grain Zn concentration and reduces grain phytate concentration, thereby increasing Zn bioavailability to humans from wheat grains (Hussain *et al.,* 2012c,d). Therefore, Zn application may be considered as a complementary approach to breeding programs focused on Zn biofortification of wheat grains. Moreover, there exists the additional benefit of higher grain yields of wheat produced on Zn deficient soils of southern Punjab.

Conclusions: The results indicated widespread soil Zn deficiency and Zn deficiency hazards for the human population consuming wheat grains produced in these soils. Nearly all the subsurface and 76% of the surface soil samples were deficient in plant available Zn resulting in low grain Zn concentration (20 $\mu g\ g^{-1}$, on average). The inherent capacity of soil to supply Zn, especially from the subsurface soil layer, positively correlated with grain Zn concentration. Low organic matter and soil salinity hazard in the zone were the other factors correlated with grain Zn concentration in wheat grown at farmers' fields. In conclusion, Zn application strategy for the zone may be considered as a complementary approach to breeding for biofortification programs. Zinc application to Zn deficient soils of southern Punjab can also ensure higher grain yield of wheat in addition to improving optimum Zn nutrition to human population.

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