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ZINC FRACTIONS IN DIFFERENTLY-TEXTURED SOILS OF SOUTHERN PUNJAB (PAKISTAN) AND STATUS OF ZINC IN FLAG LEAVES OF WHEAT

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Determining zinc (Zn) distribution in calcareous soils, is important for efficient and effective management of Zn fertilizer for optimum crop yield and quality food. The study was conducted to analyze the relationships among DTPA-extractable Zn and chemical Zn fractions in soils, important soil properties, and leaf Zn status in wheat. Seventy-five wheat fields of southern Punjab were sampled for soils (depth of 0–30 cm) and flag leaves at early heading stage of wheat. All of the sampled soils were alkaline-calcareous in nature and the soils were low in DTPA-Zn, ranged from 0.2 to 1.03 mg kg^{-1}, and variable in soil textural classes. Relative proportion of Zn fractions present in the soil-textural-class groups was in the order: structural Zn > iron (Fe) -manganese (Mn) oxide bound Zn > carbonate bound Zn > organically bound Zn >exchangeable Zn. The corresponding average Zn concentration in wheat flag leaves ranged from 12 to 18 mg Zn kg^{-1}. Leaf Zn was significantly correlated with DTPA-extractable Zn, exchangeable Zn, structural Zn, clay contents and soil organic matter contents in soils. Soil texture determined the Zn distribution in different forms. Therefore, soil texture is an important factor in measuring the plant Zn status. Hence, management of Zn fertilizer should be managed accordingly.
Keywords: calcareous soils, physicochemical properties, zinc-fraction management, fertilizer.

INTRODUCTION

Zinc (Zn) plays major role in physical, chemical and biological features in humans, soils and plants. In humans, low Zn causes mental weakness, skin discoloration, loss of taste buds, lack of appetite and irregular body growth (Prasad, 2014). It plays a significant role in proteins stability and transcription processes and is a cofactor and the only metal in nature which is the constituent of more than 300 enzymes (Chasapis *et al*., 2012).
Low soil-Zn is especially a problem of alkaline-calcareous soils of arid and semi-arid regions (Alloway, 2009). Low organic matter, low total Zn contents, sandy texture, and larger amounts of carbonate and clay contents are some factors of low Zn availability from soils. Low Zn availability from soils causes Zn deficiency in plants that correlates with Zn deficiency in humans.
Applied Zn undergoes different chemical, physical and biological changes and availability of soil applied Zn for plants uptake is decreased with time. This is because soils contain surfaces with negative charges (on clay, organic matter and Fe+Al oxides) which sorb Zn (Havlin *et al*., 2016). The knowledge of different chemical fractions, which are present in solid or solution phase of soils, is necessary to understand the Zn replenishment and bioavailability processes in soils. In active soil, Zn is distributed in different chemical forms as, attached to exchangeable planes, organic matter bound, carbonate bound and Fe-Mn oxide bound Zn (Hinsinger, 2001). Availability of Zn from these pools in soils determine plant Zn uptake (Zhang and Ke, 2004). Exchangeable Zn and organically bound Zn are readily available, amorphous sesquioxide Zn is somewhat available, and Zn in residual form is not available to plants (Keram, 2013).
For wheat, soil DTPA-extractable Zn of 1.0mg kg^{-1} is considered critical soil Zn below which Zn fertilizer application is recommended (Rashid and Ahmad, 1993). However, Zn availability to plants is a complex phenomenon and it may depend on a number of soil properties and constituents. Defining a relationship among plant Zn status, soil extractable Zn, soil properties and soil Zn pools may provide a useful information about contribution of individual Zn pools to plant availability. Therefore, the objectives of the study were (1) to determine the native Zn distribution in soils of south Punjab areas with diverse physicochemical properties, (2) to determine the Zn in different soil fractions, and (3) to correlate soil physicochemical properties, soil zinc fractions and leaf Zn concentration.

MATERIALS AND METHODS

***Soil and leaf samples*:** At early heading stage of wheat, random composite soil samples (n=75) and flag leaves (from 20 tillers at each location) were collected (depth of 0–30 cm) from wheat fields in southern Punjab, Pakistan. The soil samples in polyethylene zip-bags and leaf samples in paper bags were transported to laboratory for analysis. At laboratory, the leaf samples were dried in hot-air oven and, the soil samples were air dried. The soil was crushed to pass through a 2.0mm sieve.

***Properties of soils*: S**oil samples were analyzed for various physico-chemical properties. Soil pH was measuredin1:1 soil to water suspension and electrical conductivity (EC) in the filtrate of 1:1 soil to water suspension. Soil organic matter was determined by Walky-Black method (Walkley and Black, 1934) and acid dis solution method was followed for the determination of total calcium carbonate (Nelson, 1982). Particle size analysis (relative proportion of sand, silt and clay) was based on Hydrometer method (Bouyoucos, 1951). Soil textural classification was as described by USDA (Soil Survey Division Staff, 2017). Plant available Zn in soils was extracted by diethyl triamine Penta acetic acid (DTPA) (Lindsay and Norvell, 1978) followed by Zn determination using an atomic absorption spectrophotometer (240FS AA, Agilent, USA).

***Zinc fractions in soils*:** Total Zn in soil was estimated by the digestion in hydrofluoric (HF) and perchloric ($HClO_4$) acid. Total Zn in the soils was further fractionated sequentially into exchangeable Zn [extracted by using 1*M*$MgCl_2$ at pH7.0, carbonate bound Zn [extracted by using 1 *M*CH_3COONa at pH5.0], Fe-Mn oxide bound Zn [extracted by using 0.04 *M* NH_2OH-HCl in 25% CH_3COOH], organically bound Zn [extracted by0.02 *M* HNO_3 and 30H_2O_2 at 2.0, and then 3.2 M $CH_3COONH_4$20% HNO_3] and structural occluded Zn as difference of total Zn and sum of all other fractions (Tessier *et al*., 1979). Zinc concentration in the soil digests and extractions was measured using an atomic absorption spectrophotometer (240FS AA, Agilent, USA).

***Leaf Zn*:** Dried and ground leaf samples were digested in a mixture of HNO_3:$HClO_4$ in 2:1 ratio *(Uddin et al.*, 2016). Zinc in digests was measured using an atomic absorption spectrophotometer (240FS AA, Agilent, USA).

***Statistical Analysis*:** Statistical analyses were run on Statistix 9® for Windows (Analytical Software, Tallahassee, USA). Data were subjected to one-way ANOVA followed by pairwise comparison with Tukey's HSD test at $P \leq 0.01$ (Steel *et al*., 1997). Correlations (Pearson) were measured among physico-chemical properties, soil Zn fractions and leaf Zn concentration.

RESULTS

***Soil properties*:** Most of the soils were loams (37%) and sandy loams (37%) in texture (Table 1). All soil-textural-class groups were alkaline in nature with a mean pH range from 7.7 to 8.1.A statistically similar pH levels were observed in all the soil-textural-class groups, except loamy fine sands and sandy loams which had relatively lower pH. On average, a statistically similar level of EC was observed in the soil-textural-class groups except silty loams, which had relatively high EC. Average contents of organic matter in the soil-textural-class groups ranged from 0.2 to 1.0%. On average, clayshad5-fold higher organic matter contents than loamy fine sands. Organic matter contents had significant and positive relationship with all soil properties except sand contents. On average, calcium carbonate contents in all the soil-textural-class groups were>5%.

***Soil zinc pools*:** The total Zn contents in the soil-textural-class groups, on average, varied from 43 to 120 mg Zn kg^{-1} (Table 2). Clays were dominant in total Zn and had about 3-fold more total Zn than loamy fine sands which had lowest total Zn. Among different soil-textural-class groups, however, a statistically similar level of total Zn was observed in clays, clay loams and sandy clay loams. A major portion of total Zn in the soils was constituted as structural Zn (about 88% on average). The distribution of total Zn in structural and other Zn fractions was mainly correlated with soil texture, soil pH, soil organic matter and calcium carbonate (Table 3).

Exchangeable Zn was found to be the least fraction of Zn in the soils (Table 2). It ranged in the soil-textural-class groups from 0.18 to 0.30 mg Zn kg^{-1}. On average, clays had 66%

Table 1. Selected physico-chemical properties of differently textured soils collected from three cities of southern Punjab.

Soil-textural-class groups	n	EC (dS m^{-1})	pH	Organic Matter (%)	Calcium carbonate (%)
Clays	3	1.3±0.3b	8.0±0.06a	1.0±0.08a	6.7±0.6a
Clay Loams	8	1.4±0.4b	8.0±0.10a	0.8±0.11b	6.0±0.6ab
Silt Loams	4	1.7±0.3a	8.1±0.10a	0.6±0.12c	5.1±1.1c
Loams	28	1.3±0.4b	7.9±0.15a	0.6±0.14c	5.8±0.7bc
Sandy Clay Loams	2	1.2±0.3b	8.1±0.17a	0.7±0.05bc	6.8±0.2a
Sandy Loams	28	1.2±0.3b	7.8±0.15b	0.4±0.13d	5.8±0.4bc
Loamy Fine Sands	2	1.3±0.2b	7.7±0.01b	0.2±0.02e	5.4±0.1bc

Given values are means ± standard deviations. For each soil, means followed by different letters are significantly ($P \leq 0.01$) different from each other according to Tukey's HSD test

Table 2. Plant available zinc, total zinc and its fractions in soils, and leaf zinc of different textured soils from three cities of south Punjab.

Soil-textural-class groups	n	Total Zn (mg kg^{-1})	Exchangeable bound Zn (mg kg^{-1})	Carbonate bound Zn (mg kg^{-1})	Iron-manganese oxide bound Zn (mg kg^{-1})	Organic bound Zn (mg kg^{-1})	Structural bound Zn (mg kg^{-1})
Clays	3	120±1.6a	0.30±0.01a	3.6±0.2a	6.6±0.9cd	0.72±0.1b	109±1.5a
Clay Loams	8	107±8.3a	0.27±0.02b	3.3±0.3a	7.5±2.5b	0.69±0.2de	95±6.8b
Loams	28	87±0.2c	0.26±0.03bc	2.6±0.5b	7.1±2.2bc	0.47±0.2f	76±8.3d
Silt Loams	4	94±5.5bc	0.25±0.04cd	2.1±0.4c	10.1±2.8a	0.63±0.2e	80±5.5cd
Sandy Clay Loams	2	102±6.3ab	0.24±0.05de	3.2±0.1ab	5.9±1.4de	0.71±0.0bc	92±4.7bc
Sandy Loams	28	66±11.5d	0.23±0.02e	3.0±0.4ab	5.7±1.7e	0.70±0.2cd	56±10.3e
Loamy Fine Sands	2	43±0.0e	0.18±0.02f	3.1±0.2ab	2.5±1.7f	0.77±0.0a	36±1.4f

Given values are means ± standard deviations. For each soil, means followed by different letters are significantly ($P \leq 0.01$) different from each other according to Tukey's HSD test

Table 3. Values of significant Pearson correlation coefficients (r) for soil physicochemical properties, soil zinc fractions and leaf Zn concentration.

Variables	Sand	Silt	Clay	Organic Matter	DTPA-Zn	Exchange-able Zn	Fe-Mn oxide bound Zn	Organic matter bound Zn	Structural Zn
Sand	1.00	−0.82	−0.64	−0.82	−0.70	−0.63	−0.44	−0.90	−0.90
Silt		1.00		0.52	0.67	0.45	0.51	−0.36	0.59
Clay	−0.64		1.00	0.72	0.31	0.51			0.77
Organic Matter	−0.82	0.52	0.72	1.00	0.70	0.532	0.54	0.09	0.93
Calcium Carbonate			0.38	0.37					0.27
pH	−0.56	0.44	0.37	0.42	0.07	0.26	0.25	−0.25	0.67
DTPA-Zn	−0.70	0.67	0.31	0.70	1.00	0.55	0.74		0.60
Fe-Mn oxide bound Zn	−0.44	0.51		0.54	0.74		1.00	0.32	0.48
Carbonate bound Zn	−0.90	0.59	0.46	0.26				0.51	
Structural Zn	−0.90	0.59	0.77	0.93	0.60	0.61	0.48		1.00
Leaf-Zn	−0.33		0.35	0.30	0.52	0.33			0.23

Only significant ($P \leq 0.01$) correlations are shown

higher exchangeable Zn than loamy fine sands. Average contents of organic matter bound Zn ranged from 0.47 to 0.77 mg Zn kg^{-1} in the soil-textural-class groups and these were 2.6-foldshigher than exchangeable Zn. A statistically similar contents of organic-occluded Zn were observed in clay loams, sandy clay loams and sandy loams. Onaverage, about1.6-foldshigherorganic matter bound Zn was present in loamy fine sands than loams. On average, carbonate bound Zn was4.5-folds higher than organic bound Zn and ranged from 2.1 to 3.6 mg Zn kg^{-1} in the soil-textural-class groups. On average, a statistically similar level of carbonate bound Zn was observed in clays and clay loams. Contents of carbonate bound Zn were also statistically similar in loamy fine sands, sandy clay loams and sandy loams but lower in loams and silt loams.

On average, Fe-Mn oxide bound Zn was the second most dominant fraction in soil-textural-class groups after structural Zn;it was about 2-foldshigherthan carbonate bound Zn (Table 2).The contents of Fe-Mn oxide bound Zn distributed variably among soils and ranged from 2.5 to 10.1 mg Zn kg^{-1}. On average, silt loams had highest Fe-Mn oxide bound Zn than other soil-textural-class groups.

***Soil and LeafZn status*:** Average DTPA-extractable Zn in the soil-textural-class groups ranged from 0.2 to 1.0 mg Zn kg^{-1} (Figure 1). On average, a statistically similar level of DTPA-extractable Zn was observed in clays, clay loams, loams and silt loams; but significantly lower in loamy fine sands, sandy clay loams and sandy loams. Clays had 4.7-folds higher DTPA-extractable Zn, on average, than loamy fine sands. DTPA-extractable Zn in soils had positive significant relationship with exchangeable Zn, Fe-Mn oxide bound Zn, structural Zn, clay contents, silt contents, soil organic matter, and soil pH (Table 3).

Average leaf Zn concentration in wheat grown on different soil-textural-class groups ranged from 12 to 18 mg Zn kg^{-1} (Figure 2). On average, a statistically similar level of leaf Zn was observed in wheat grown on loams, sandy clay loams, sandy loams and silt loams which were 82% of the samples fields. Leaf Zn was 1.3-folds higher in wheat grown on clays than loamy fine sands. Leaf Zn was positively correlated with

DTPA-extractable Zn, exchangeable Zn, structural Zn, clay contents and soil organic matter; but negatively correlated with sand contents in soils (Table 3).

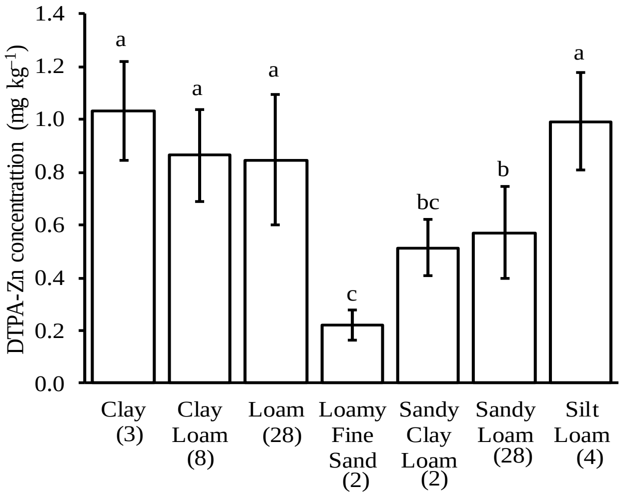

Figure 1. Extracted zinc with 0.005 M DTPA in soils belonging to seven textural classes of three cities of southern Punjab.

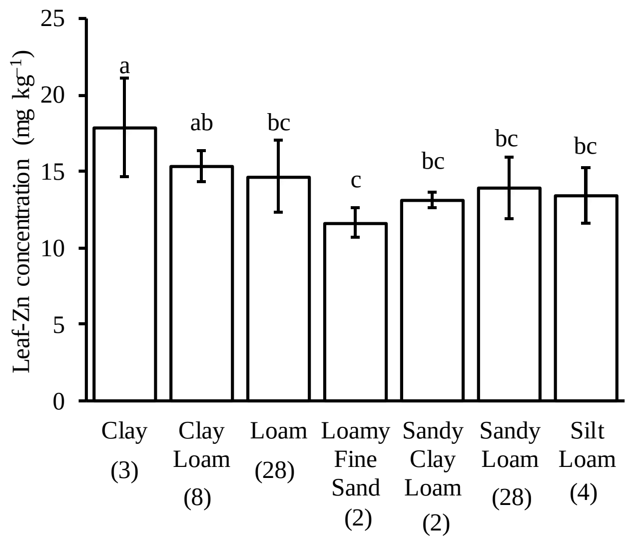

Figure 2. Concentration of leaf Zn in soils belonging to seven textural classes of three cities of southern Punjab.

DISCUSSION

The studied soils were alkaline-calcareous in nature, low in organic matter and variable in texture (Table 1). These soil characteristics relate with semi-arid climatic conditions with low annual precipitation (which led to less leaching of basic cations) and consistent high temperature (which causes rapid decomposition of organic matter), and calcareous parent material (Lehmann and Kleber, 2015; Mccauley *et al*., 2017). The average total Zn in the studied soils (Table 2) was within the range reported in calcareous soils of Pakistan (Hussain *et al*., 2011). The ranges of Zn in soil Zn fractions observed in the study (Table 2) are in agreement with previous reports (Kamali*et al.*,2010) who also obtained highest Zn concentration in the structural fraction and lowest in exchangeable fraction. The greater percentage of structural Zn among fractions indicated greater tendency of Zn to become unavailable to plants (Ramzan *et al*., 2014). Structural Zn was higher in clays but lower in loamy fine sands (Table 2), indicating that structural Zn was mainly entrapped in silicate clays (Refaey *et al*., 2017).

Exchangeable Zn was very low in soils (Table 2) mainly because of sandy nature of some of the soils, presence of $CaCO_3$, unfavorable high pH and low organic matter content in soils (Hussain *et al*., 2011). Other reasons could be high buffering capacity of soils and adsorption of Zn on soil surfaces (Robarge, 2018). The alkaline-calcareous soils have hydroxyl and oxide groups, and insoluble Ca compounds which convert available Zn to unavailable forms as $Zn(OH)_2$ and $CaZnO_2$ (Mahmoud Soltani *et al*., 2015). Clays were dominant in this fraction as compared to other soil-textural-class groups mainly due to high surface area and high cation exchange capacity (Khosh go ftarmanesh *et al.*, 2018). Organically occluded Zn was also lower in the soils (Table 2) particularly due to lower contents of soil organic matter in soils, and a high decomposition rate of organic matter due to elevated temperature of the region (Preetha and Stalin, 2014).As in the present study (Table 2), Fe-Mn oxide bound Zn dominates in non-residual fractions (Prashantha, 2011).

The positive significant relation of exchange able Zn, Fe-Mn oxide bound Zn, structural Zn, clay, silt, organic matter, pH and leaf Zn with DTPA-Zn (Table 3) may link with low availability of soil Zn (Figure 1). DTPA-extractable Zn was significantly lower in soils due to low Zn buffering capacity, less exchangeable sites and unavailability of Zn minerals in the soils (Noulas *et al*., 2018). Low DTPA-Zn is the characteristic of the area as, many other researchers (Hussain *et al*., 2011) also reported low Zn in calcareous soils of Pakistan.

According to Rafique *et al*. (2006), critical average leaf Zn concentration in flag leaves of wheat ranges from 12 to 16mg kg^{-1}; below this, grain yield is limited by low Zn availability. Leaf Zn concentration in most of the wheat fields (Figure 2) was within the recommended the critical level (Rafique *et al*., 2006). Cumulative effect of alkaline pH, calcareousness, low organic matter (Table 1) low availability of Zn minerals in the soils (Table 3) may link with low Zn in leaves (Figure 2).

***Conclusion*:** It can be concluded from the given study that, in southern Punjab, soil texture determines the distribution of Zn in different forms. Hence, plant Zn availability measured as plant Zn status is dependent on soil texture. So, Zn fertilizer application may be managed accordingly.

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