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INFLUENCE OF TIME ON RELEASE BEHAVIOUR OF ZINC AND ITS FRACTION IN DIFFERENT PHOSPHORUS STATUS SOILS

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ABSTRACT

Zinc in soil exists in different chemical forms, while its total concentration does not provide accurate information about its bioavailability, the application of fractionation approach provides information on the metal's actual and potential availability. In this study, an incubation experiment was conducted to evaluate the influence of time on DTPA-extractable Zn and Zn fractionation in two different phosphorus status soils. The study's findings show that in both kinds of soil, the release of DTPA-extractable Zn and all other Zn fractions consistently decreased during the course of incubation. Although the pattern of DTPA-extractable Zn release and its fractions remained same but their amounts were noticeably different across the two soils, reflecting their varying capacities for continuous supply of Zn to the soil solution. Based on the results, comparison of the values shows that the concentration of DTPA-extractable Zn and its fractions for high P status soil was more than low P status soil. Moreover, Zn fractions followed the same order for both the soils, *viz*. RES-Zn > Fe/MnOX-Zn > CAR-Zn > ORG-Zn> WSEX-Zn. Among the fractions in both the soils, RES-Zn was the dominant fraction that contributed most to the total-Zn, followed by Fe/MnOX-Zn, whereas, the contribution of WSEX-Zn fraction to the total-Zn was relatively low.
***Keywords*:** Release behaviour, Zn fractionation, incubation, phosphorus status

Introduction

The deficiency of micronutrient has become a major limiting factor for crop productivity in many parts of the world more specifically in India due to sole application of macronutrients without supplementing micronutrients or manures. Among the micronutrients' deficiencies, zinc (Zn) deficiency is considered to be the most ubiquitous in many parts of the world (Dinesh *et al*., 2018). This is because of the fact that Zn is the only micronutrient relevant to almost all classes of enzymes in biological systems (Broadley *et al*., 2007) and about 2800 proteins require Zn for their structural integrity and activity (Andreini *et al*., 2009). Despite these facts that zinc is recognised to be crucial for plants, animals and humans, Zn deficits still affecting today also (Obrador *et al*., 2003). As one-third of the world's population is currently experiencing serious nutritional and health issues due to the Zn deficiency in millions of hectares (Myers *et al*., 2015; Dinesh *et al*., 2018). This is because of the fact that Zn deficiency not only reduces yields of crops but also severely impairs nutritional quality of produce. The main soil factors affecting the availability of Zn to plants are low Zn contents in soil, high pH, high calcite, high organic matter content, sesqui-oxides and high concentration of Na, Ca, Mg, bicarbonate and phosphate in soil solution or in labile forms (Alloway, 2009; Baghernejad *et al*., 2016). Zinc availability in soil increased with the decrease in soil pH. Several reports demonstrate that Zn tends to be less available to the plants in the soil having high pH. Low soil pH reduced Zn adsorption in sandy soils as compared to those for high in colloidal-size materials (Veni *et al*., 2014). Moreover, zinc deficiency occurs in soil with a low total Zn content (*i.e.* sandy soil) or soil which has relatively high available phosphorus content (Singh *et al*., 2005). Also, the soil having high clay content or organic matter had greater adsorptive capacity for Zn as compared to sandy soil (Lorenz *et al*., 2000; Veni *et al*., 2014). High soil P can affect soil properties, which in turn will influence the availability of zinc to the

plants. Phosphorus fertlizers, when applied to the soil, can change pH of the soil due to either its dissolution effect or due to the reaction of both the P and associated cations with soil components. Phosphorus can also change surface charge of the soil due to the adsorption of P on colloids and direct precipitation of Zn with P. Changes in chemical properties brought about by phosphate additions can alter the equilibrium of Zn in the soil, leading to a redistribution of Zn in different soil fractions (Tagwira *et al*., 1992). The availability of Zn is also affected by moisture and temperature. Zinc deficiency observed in cool and wet soil during early growing season if temperature is rather low (Noulas *et al*., 2018). On the other hand, increased soil temperature increases Zn supply, diffusion rate from soil colloids to plant roots and its mineralization rates from organic matter. Though, it is generally considered that residence time effects reduce metal mobility and bioavailability, very few studies have been conducted to determine whether and how time effects affect the metal chemical forms and/or metal fractions in soil (Lim *et al*., 2002; Davies *et al*., 2003; Lu *et al*., 2004). The length of contact period between applied Zn and the soil or soil constituent can have an important influence on the bioavailability of Zn (Barrow, 1986). The availability of applied Zn to plants in Zn deficient soil declined with increasing time of contact between soil and the applied Zn (Armour *et al*., 1989). However, total Zn content of soil is not a reliable index to reflect the capacity of soil to supply Zn for plant uptake. A very small part of the total soil Zn (< 1 mg kg^{-1}) is present in the soil solution, when can be taken up by the crops (Kabata-Pendias, 2000). Zinc in soil exits in different chemical forms like water soluble, exchangeable, organically bound, manganese oxide bound, amorphous oxide bound, crystalline oxide bound and residual fractions (Mandal and Mandal, 1986), thus affects its availability to crops. Therefore, a better understanding of Zn behaviour in relation to the transformation of Zn added to soil is provided by the distribution of Zn in different soil fractions (Lyengar *et al*., 1981). The relative amount of each of these Zn forms differs considerably with soil type and can be affected by various soil physical and chemical properties (Tagwira *et al*., 1992). Therefore, the objectives of this study were to determine DTPA- extractable Zn and to determine the release behaviour of different forms of Zn in different P status (low and high) soils at different incubation time (days).

Materials and Methods

Soil sampling, preparation and analysis

The soils used in the present investigation was collected from the farmer's fields at two different locations of Haryana, *viz.* Sadalpur (Hisar) and Saniyana (Fatehabad). Soil collected from village Saniyana was high in available P, whereas, that collected from village Sadalpur was low in available P content. The application of phosphatic fertilizers was being practiced by the farmers in both types of soil since many years. These soil samples were brought to the laboratory, air dried, crushed and sieved through 2 mm sieve and then subjected to various physico-chemical properties analysis by using standard methodology. The sand, silt and clay fractions were determined by international pipette method (Piper, 1966), pH and electrical conductivity (EC) in soil: water (1:2) suspension was determined by using pH and EC meter, respectively. Soil organic carbon content was calculated by adopting standard procedure of Walkley and Black (1934), Cation exchange capacity was determined by the displacement of cations with ammonium acetate by using the method of Hesse (1971), the available N was determined by Kjeldahl method (Subbiah and Asija, 1956), available P by Olsen'method (Olsen *et al*.,1954), available K by using a flame photometer and diethylene-triamine-pentacetic acid (DTPA)- extractable zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) were extracted by using DTPA solution buffered at pH 7.3 (Lindsay and Norvell, 1978). The values of initial physico-chemical properties of both the studied soils have been presented in Table 1.

Table 1 : Initial physico-chemical properties of low and high phosphorus status soils

Initial soil parameters	Low P staus soil	High P status soil
pH	7.4	7.1
EC (dS m^{-1})	0.12	1.0
Sand (%)	92	60
Silt (%)	4	24
Clay (%)	4	16
Texture	Sand	Sandy loam
CEC [(cmol (p^{+}) kg^{-1})]	4.46	9.28
Organic carbon (%)	0.15	0.62

Available nitrogen (kg ha^{-1})	28	182
Available phosphorus (kg ha^{-1})	8	25
Available potassium (kg ha^{-1})	112	430
DTPA-extractable zinc (mg kg^{-1})	0.30	0.72
DTPA-extractable copper (mg kg^{-1})	1.94	3.08
DTPA-extractable iron (mg kg^{-1})	2.11	18.74
DTPA-extractable manganese (mg kg^{-1})	2.48	10.26

Incubation Study

An incubation study was conducted by placing 100 g of both types of soils (*i.e.* low and high P status soils) saparately at field capacity in wide mouth plastic bottles and replicated thrice. The moisture content in these soils samples was maintained periodically by weighing the bottles. And the loss in weight of bottles was adjusted by adding distilled water, and bottles were again placed in the incubator. Both the soils were incubated at ambient temperature (25 ± 2 °C) under laboratory conditions for 1, 7, 14, 21, 28 and 35 days. After each incubation interval, the soil samples were taken out from the incubator, air-dried, crushed and sieved. The DTPA extractable Zn was estimated by using DTPA solution buffered at pH 7.3 (Lindsay and Norvell, 1978) and Zn fractions were estimated by following sequential extraction procedure of Tessier *et al*. (1979) and modified by Jeng and Singh (1993). Extractions were performed in 50 ml polypropylene centrifuge tubes and between each successive extraction, the supernatant was centrifuged at 5000 rpm for 30 min, filtered and their contents were determined by using Atomic Absorption Spectrophotometer (Model: Varian AA240z). For determination of total-Zn, 1 g of soil was digested with diacid mixture (HNO_3 and $HClO_4$ solution). The digested samples of both the soils were filtered and the total-Zn content was determined by using atomic absorption spectrophotometer. The extraction conditions and the chemical reagents used for corresponding fractions are listed in Table 2.

Table 2 : The chemical reagents and conditions for the sequential extraction procedure of different zinc fractions

Fractions	Reagent	Extracting conditions
F_1: Water soluble plus exchangeable (WSEX-Zn)	1g of soil sample and 20 ml of 0.5 M Ca $(NO_3)_2$	Shake for 16 hours
F_2: Carbonate bound (CAR-Zn)	8 ml of 1M NaOAc (pH 5)	Shake for 6 hours
F_3: Fe/Mn oxides bound (Fe/MnOX-Zn)	20 ml of 0.04 M $NH_2OH.HCl$ in 25% acetic acid	Heat on water bath (96^0C) for 6 hours
F_4: Organically bound (ORG-Zn)	3 ml of 0.02 M HNO_3, 5 ml of 30% H_2O_2 (pH 2), 5ml of 3.2 M NH_4OAc in 20% HNO_3 was added after boiling on water bath	Heat on water bath (85^0C) for 2 hours and shake 30 minutes
F_5: Residual (RES-Zn)	Concentrated H_2SO_4 (4-5 drops), 5 ml of 48% HF and 1 ml of 60% $HClO_4$	

Results and Discussion

DTPA-extractable Zn

It is evident from the trend shown in Figure 1 that the DTPA-extractable Zn content in both the soils decreased gradually with the progress of the incubation period. The DTPA-extractable Zn content decreased from 0.34 to 0.21 and 0.76 to 0.60 mg kg^{-1} in low and high P status soils, respectively from 1 to 35 days after incubation (DAI). Initially, the rate of release of DTPA-extractable Zn was found higher in both the soils but later on it decreased with the increase in period of incubation. Comparing both types of soils, the amount of DTPA-extractable Zn was higher in high P status soil as compared to low P status soil. This might be due to the difference in their initial Zn content of respective soil, difference in organic carbon content and CEC. Singh *et al.* (2006), Talukder *et al*. (2011) and Ghasemi-Fasaei *et al*. (2012) also reported that longer the contact time of Zn with soils resulted in the lesser desorption of both native and added Zn. The observations of this study also agree with the findings of Kuo and Mikkelson (1980) who also showed a decrease in amount of Zn desorbed by DTPA with increasing aging time, but this was in contradictory to the findings of Dey *et al*. (2019) who reported consistent increase in Zn release from different organic amendment throughout the incubation period.

Zinc fractions

Figure 2 and 3 depict the time dependent changes in content of each fraction of zinc and distribution of Zn in different fractions including water soluble exchangeable-Zn (WSEX-Zn), carbonate-Zn (CAR-Zn), iron and manganese-Zn (Fe/MnOX-Zn), organic-Zn (ORG-Zn) and residual-Zn (RES-Zn) in both types of soil incubated for 1, 7, 14, 21, 28 and 35 days. It was recorded that in both the soils, the content of aforementioned Zn fractions decreased gradually with the increase in incubation period, *i.e*., from 1 to 35 days. The distribution of different Zn fractions in both soils was found in order: RES-Zn > Fe/MnOX-Zn > CAR-Zn > ORG-Zn > WSEX-Zn. Among both types of soil, the release in the content of different forms of Zn at all incubation period was high in high P status soil as compared to low P status soil. Zahedifar (2017) reported similar distribution pattern of Zn fractions in soils of different land use types. Lu *et al*. (2004) also investigated the time effect on fractionation of heavy metals in soils and found that Cu, Zn, Pb and Cd in each fraction were generally in the order residual > exchangeable > Fe-Mn oxides > organic matter > carbonate fraction in three different textured soils. Compared among the soils, changes of metal fractions in Jiangxi soil were much slower than in other two soils, which were mainly attributed to the low soil pH, and the soil with high organic matter content could bind more metals in organic matter fraction and this was also in agreement with the findings of Martinez and McBride (2001).

Changes in water soluble/exchangeable-Zn

In both the soils greater concentration of WSEX-Zn was found after one day of incubation and it decreased as incubation time was prolonged. After one day of incubation, the Zn in this fraction was 1.70 and 2.85 mg kg^{-1} in low and high P status soils, respectively. After the incubation time of 7 to 28 days, the concentration of WSEX-Zn fraction decreased from 1.64 to 1.39 and 2.85 to 2.69 mg kg^{-1} in low and high P status soils, respectively. After 35 days of incubation the Zn content decreased to 1.21 and 2.46 mg kg^{-1} for low and high P status soils. However, the content of Zn in WSEX fraction at all the incubation periods was comparatively higher in high P status soil than low P status soil. The results of present study corroborate with the findings of Lu *et al*. (2005) who showed that Zn in exchangeable fraction decreased with increasing incubation time. In this study, the contribution of WSEX-Zn fraction to the total-Zn was least among all the fractions in both the soils. Preetha and Stalin (2014) also reported similar results and stated that high buffering capacity of these soils resulted in low amount of water soluble + exchangeable Zn.

Changes in carbonate bound- Zn

Similar to the WSEX-Zn, the release of CAR-Zn was higher at the start of the incubation, *i.e*., at 1st day of incubation and thereafter declined consistently with the progress in incubation period in both kinds of soil. From 1st to 35 days of incubation, the content of CAR-Zn decreased from 3.16 to 2.87 mg kg^{-1} in low P soil and 4.06 to 3.67 mg kg^{-1} in high P status soil. The content of Zn in this fraction in low P status soil was comparatively lower than the high P status soil. These results were in contradictory to the findings of Zahedifar (2017). Lu *et al*. (2005) reported that the concentration of Zn in this fraction decreased with the increase in incubation period in Jiangxi soil but in Beijing and Heilongjiang, Zn increased with the increase in incubation period.

Changes in iron and manganese bound Zn

Similar to the WSEX-Zn and CAR-Zn, the Fe/MnOx-Zn fraction also decreased with the increase in incubation period in low as well as in high P soils. It has been observed that the highest Zn content in this fraction in low and high P soil was 8.60 and 10.03 mg kg^{-1}, respectively, which was found on 1st day of incubation. With increasing incubation period from 7 to 28 days, the Fe/MnOx-Zn content decreased from 8.57 to 8.47 mg kg^{-1} in low P and 10.00 to 9.87 mg kg^{-1} in high P status soil. On 35 days of incubation, the content of Zn in this fraction reached to a value of 8.26 and 9.69 mg kg^{-1} in both low and high P status soils, respectively. The results recorded in the present study were contradictory with the findings of Lu *et al*. (2005) who reported that Zn in this fraction increased with increasing incubation time. This fraction was the second most prevalent among all the Zn fractions right after RES-Zn. Similar findings were also reported by Preetha and Stalin (2014). Figure 2 and 3, demonstrate the concentration of Zn in Fe/MnOx-Zn was greater in high P status soil than in the low P status soil on each day of incubation. Higher content of this fraction in high P status soil than the other soils might be due to higher content of sesquioxides, which attributed to higher adsorption of Zn due to more specific surface area (Preetha and Stalin, 2014 and Wijebandara *et al*., 2011). According to Filgueiras *et al*. (2002), weathering may mobilize heavy metals associated with this fraction. Asada *et al*. (2012) reported that clay minerals have high adsorption attraction of exogenous Zn.

Changes in organically bound Zn

The amount of Zn bounded with organic matter also decreased consistently with increasing incubation periods. The highest and lowest content of Zn in this fraction were 2.06 and 1.61 mg kg^{-1} in low P status soil and 3.18 and 2.80 mg kg^{-1} in high P status soil, respectively, which were recorded on 1 and 35 days of

incubation. The increased in Zn content in this fraction in high P status soil was more in the low P status soil. This could be due to the relatively more organic carbon content of the high P status soil. According, to Lu *et al.* (2005), the soil with high organic matter content could bind more metals in organic matter fraction. They also reported that Zn content in organically bound fraction in three different soils increased as the incubation time prolonged, which was contradictory with the results of present study. Similar to the WSEX-Zn, this fraction also contributed less to total-Zn and this could be due to low to medium organic matter content in both the soils. These findings are in agreement with the findings of Preetha and Stalin (2014).

Changes in residual Zn

It is evident from Figure 2 and 3 that similar to aforementioned Zn fractions, the residual-Zn content consistently decreased with the progress in incubation period from 1 to 35 days in both types of soil. The highest Zn content in low and high P status soils were 27.40 and 52.00 mg kg^{-1}, respectively, which were observed after one day of incubation. With the increase in incubation period from 7 to 28 days, the Zn in RES-Zn fraction reached to 26.39 and 51.80 mg kg^{-1} in low and high P status soils, respectively. After 35 days of incubation, the lowest Zn content in this fraction was found to be 26.10 mg kg^{-1} in low P status soil and 51.59 mg kg^{-1} in high P status soil. Also, the Zn content in this fraction on all incubation days was more in high P status soil as compared to low P status soil. It was found that most of the Zn in both the soils was present in the residual form followed by Fe/MnOx-Zn. This was in agreement with the findings of Lu *et al.* (2005). Variation in the amount of residual Zn could be due to the type of dominant clay minenals (Preetha and Stalin, 2014).

Changes in Total-Zn

The Figure 2 and 3 showed that the amount of total-Zn was greater in beginning of the incubation and decreased when the incubation time was prolonged up to 35 days. After one day of incubation, the total-Zn in low and high P status soils was 42.92 and 72.12 mg kg^{-1}, respectively, which was found to be maximum in both the soils. From the incubation time of 7 to 35 days, the amount of total-Zn reached to 40.05 mg kg^{-1} in low P status soil and 70.21 mg kg^{-1} in high P status soil. In comparison to low P status soil, the higher amount of total-Zn was observed in high P status soil, which was due to higher content of other Zn fractions in high P status soil as compared to the other soil. Ratuzny *et al.* (2009) reported that the distribution of heavy metals between different fractions is dependent on their source and soil characteristics.

Conclusion

It was observed that the release of DTPA-extractable Zn and all of the Zn fractions decreased gradually with the increase in incubation period in both types of soil. The highest and lowest content of DTPA-extractable Zn and its fractions were observed after 1 and 35 days of incubation. The amount of DTPA-extractable Zn and its fractions differed but the release pattern of DTPA-extractable Zn and its fractions remained the same in the soils at two locations. High P status soils had greater content of DTPA-extractable Zn and its fractions as compared to low P status during 35 days of incubation. However, the order of preponderance of Zn fractions in both the soils remained same, *viz.* RES-Zn > Fe/MnOX-Zn > CAR-Zn > ORG-Zn > WSEX-Zn. Among the fractions in both the soils, RES-Zn was the dominant fraction, which contributed most to the total-Zn, followed by Fe/MnOX-Zn, whereas, the contribution of WSEX-Zn fraction to the total-Zn was lowest.

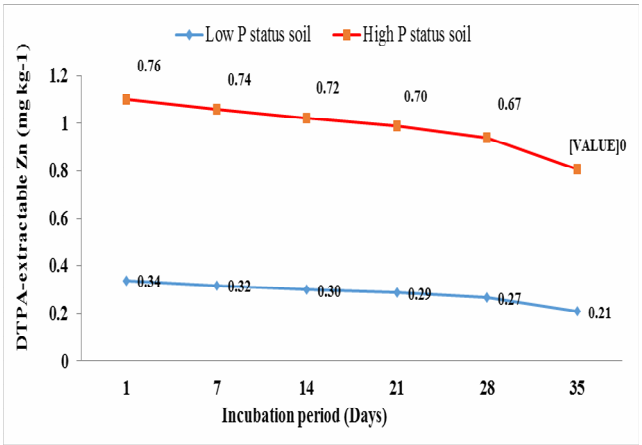

Fig. 1: Release behaviour of DTPA-extractable Zn in low and high P status soils at different days of incubation

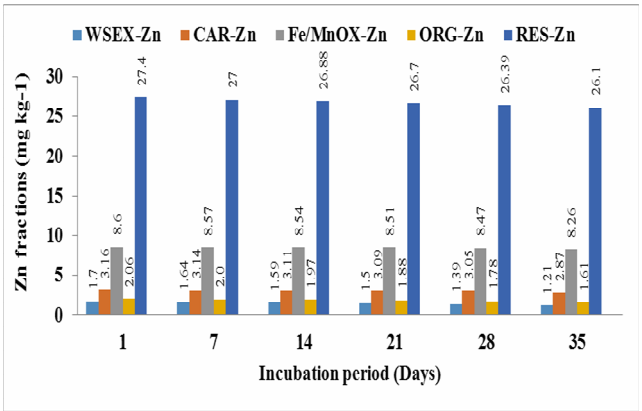

Fig. 2: Distribution of various forms of Zn in low P status soil at different days of incubation

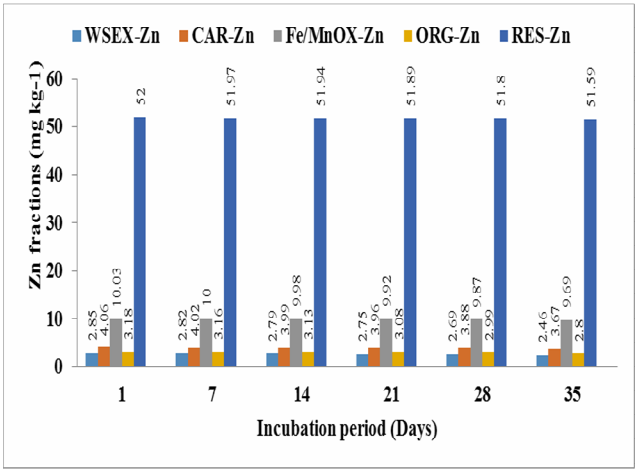

Fig. 3: Distribution of various forms of Zn in high P status soil at different days of incubation

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