Evaluation of zinc fractions as influenced by long term fertilization under rice-wheat cropping system

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Abstract
An investigation was carried out on permanent plots at IGKV, Raipur research farm during kharif 2019 in order to conduct experiment entitled “Evaluation of zinc fractions as influenced by long term fertilization under rice-wheat cropping system”. “Ten treatments for this study comprised no fertilizer “control, 50% NPK, 100% NPK, 150% NPK and (NPK) combined with ZnSO₄ (100% NPK + ZnSO₄ @ 10 kg ha⁻¹), 100% NP, 100% N, 100% NPK + FYM @ 5 t ha⁻¹, 50% NPK + BGA @ 10 kg ha⁻¹ and green manure (50% NPK + GM @ 40 kg ha⁻¹), laid out at randomized complete block design with four replications.” The different form of zinc fractions were analyzed at surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Among “various forms of zinc fractions namely, water soluble plus exchangeable Zn (WSEX-Zn), organically bound Zn (Org-Zn), amorphous sesquioxide bound Zn (AMOX-Zn), crystalline sesquioxide bound Zn (CRYOX-Zn), residual Zn and total Zn.” The data on zinc fractions revealed that the continuous application of inorganic fertilizers and organic manures improved available zinc and its fractions in surface (0-15 cm) soil. The highest “available Zn content was recorded in 100% NPK+Zn followed by 100% NPK+FYM in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Among “various forms of zinc fractions namely, water soluble plus exchangeable Zn (WSEX-Zn), organically bound Zn (Org-Zn), amorphous sesquioxide bound Zn (AMOX-Zn), crystalline sesquioxide bound Zn (CRYOX-Zn), residual Zn and total Zn.” The water soluble plus exchangeable zinc (WSEX-Zn) fraction “was found to be the least among the zinc fractions under various treatments in surface and sub soil, respectively. The residual zinc fraction contain highest amount of Zn and it was recorded in 100% NPK+Zn followed by 100% NPK+FYM in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively.” While, the lowest residual Zn content was recorded in control plot in both the surface (0-15cm) and subsurface (15-30cm) soil, respectively. The similar trend was also obtained under all the Zn fractions where the highest Zn content was recorded in 100% NPK+Zn followed by 100% NPK+FYM in surface (0-15 cm) and subsurface (15-30 cm) soils and lowest Zn content was obtained under control plot. The Zn fractions differs accordingly as WSEX-Zn < Org-Zn < AMOX-Zn < CRYOX-Zn < res-Zn.” Similarly, “organic manure addition also increased the Zn content in the surface soil. The highly positive correlation was obtained between different fractions of zinc and soil organic carbon and their combined effects on yield of rice under long term fertilizer experiment. Existing investigations show that generally the various Zn fractions increases on the surface (0-15 cm) soil, while, they decreases with the soil depth even when using Zn fertilizer.

Keywords: Zinc fractions, water soluble plus exchangeable zinc, organically bound zinc, amorphous sesquioxide bound zinc, crystalline sesquioxide bound zinc, residual zinc, long-term fertilizer

Introduction
Zinc is one of the most important micronutrients for the growth of rice grown especially in submerged conditions. Zinc deficiency distributed globally in tropical and temperate climates (Slaton et al., 2005; Prasad; 2006; Fageria et al., 2011). Zn shortage mostly occurs in developing (third world) countries; where there is an urgent need to increase food production. For achieving crop yield targets, there is need to reduce Zn deficiency in the soil, but also a poor Zn content for humans and animals as a result of low Zn content in grains and straw. (Scratt, 2006). Zn bioavailability has been reported to be associated with its alteration in soil and plant continuity through various mechanisms such as soil and surfaces, hydrosode oxide minerals, organic materials, and subsequent adsorption, which affect the zinc by crops (Soltin et al., 2015). Total Zn of soil can be classified into five fractions using sequential or batch partitioning schemes (Saffari et al., 2009).

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These fractions include a water-soluble pool, present in the soil solution, exchangeable pool with ions bound to the clay particles by electric charges, consisting of organically bonded ions, adsorbed, chelated or complexed with the organic ligand, Pool minerals of non-exchangeable pools of zinc on the soil and pools of insoluble metal oxides and weathering primary minerals (Acef, 2008). For predicting the availability of soil Zn on plant regeneration all these fractions supply comprehensive information on biological, geological and chemical processes occurring in a soil. It has been concluded that residual Zn and oxide bound Zn are more constant fractions while exchangeable Zn and water-soluble Zn fractions are more soluble and available to plants (Rahmaniet al., 2012).

Long-term fertilizer experimentation (LTFE) plays an important role in understanding complex interactions involving plant, soil, climate, management practices, and their effects on soil productivity over long periods of time (Clay et al., 2012). Despite the fact that the measure of zinc required for crop development is far not as much as that of macronutrients, Zn inadequacy in soil has been accounted for generally from various pieces of the world (Welch RM et al.,). It is realized that a great part of the Zn stay in soils bound by oxides of iron. On submergence, these oxides experience decrease due to anaerobiosis, to the lower valiant structures which are increasingly solvent. The bound Zn is in this manner discharged and opens up to the harvest plants. (Obradoret al., 2007) [13]. It has been seen that soil different Zn pools: water soluble, easily exchangeable, adsorbed, chelated, or complexed, associated with secondary minerals, and held in primary minerals. (Viets FG Jr, et al.1962). Zinc in soluble organic complexes and exchange positions are vital in keeping up of Zn fertility levels adequate for crop prerequisite. (Murthy ASP et al, 1982) [12].

Materials and Methods

Study Site Description

A long-term field experiment in rice during Kharif 2019 was conducted at the research farm of Indira Gandhi Krishi Vishwavidyala, Raipur, located at 22° 33’ N to 21° 14’ N latitude and 82° 6’ E to 81° 38’ E Longitude with the altitude of 293m above Mean Sea Level. Raipur region is sub-humid, temperatures remain moderate throughout the year, except from March to June. It receives an about 1200 - 1300mm (51”) of rain, mostly in the monsoon season from late June to early October. The soil of the experimental field was Vertisol, which was fine Montmorillonite, Hyperthermic, Chromustert, also locally called as Kanhar and identified as Arang II series, having pH (7.5), EC (0.23 ds m-1) and OC (0.60%), available N, P, K and Zn were 212, 21, 386 kg ha-1 and 1.94 mg kg-1, respectively.

Experimental details

The field experiment was conducted in randomized block design with four replications in rice as a test crop and supplementing a part of nitrogen through different organic sources viz., FYM, green manure (Sesbania aculeata) and blue green algae. A set of ten treatments was repeated in the permanent laid out plots with following treatment details: T1 – Control, T2 - 50% of the recommended optimum NPK dose, T3 - 100% of the rec. optimum NPK dose, T4 - 150% of the rec. optimum NPK dose, T5 - 100% of the rec. optimum NPK + ZnSO₄ @ 10 kg ha⁻¹ in kharif crop only, T6 - 100% NP of rec. optimum N and P dose, T7 - 100% N of rec. optimum N dose, T8 - 100% NPK + FYM (5 t ha⁻¹), T9 - 50% NPK + BGA (10 kg ha⁻¹), T10 - 50% NPK + Green manure. Use of 100% NPK corresponded to the state level recommended dose of N,P and K for respective rice variety (Rajeshwari R-1), which was 120:60:40 kg ha⁻¹ for rice, respectively. In rice, half of N and entire quantity of P and K were applied as basal dose through urea, SSP and MOP, respectively. Sesbaniaaculeata (Sunhamp) was grown as an in-situ green-manure during kharif (June to July) for a period of 45 days and was chopped into small pieces of 5-7 cm and incorporated into the soil by a power operated rotator at the time of puddling before transplanting of rice. Transplanting of rice variety IGKV R-1 (Rajeshwari) during kharif 2019 was done on 15th July.

Soil sampling and analysis

The soil samples were collected with the help of tube auger from the each plot at the surface (0-15cm) soil from the long term fertilizer experiment field at Experimental farm of Department of Soil Science and Agricultural Chemistry, Indira Gandhi Krishi Vishwavidyalayal, Raipur (C.G.) was taken before sowing of rice (May, 2019) and were analyzed for soil pH, Organic carbon and EC using standard methods of analysis and the pooled data have been presented here. However, the surface (0-15cm) and subsurface (15-30cm) soil samples taken before sowing of rice were analyzed for different fractions of zinc. The different fractions of zinc were analyzed with the standard procedure by the procedure of Murthy (1982) [12]. The flow chart of the sequential fractionation scheme is shown below:

a. Water soluble plus exchangeable Zn
b. Organically bound Zn
c. Amorphous sesquioxide bound Zn
d. Crystalline sesquioxide bound Zn
e. Residual Zn
f. Total Zn

Results and Discussions

Zinc fractions

The available Zn in soil significantly increased with effect of continuous application of inorganic fertilization and organic manures. Highest (1.94 mg kg⁻¹) and (0.94 mg kg⁻¹) available Zn content were recorded in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soils respectively. While the lowest (0.67 mg kg⁻¹) and (0.45 mg kg⁻¹) available Zn content were recorded in control at both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Similar results were reported on the available Zn content revealed that there was a high addition of Zn with 100% NPK + FYM even though with Zn use"was found to be high.” In decisive examination of the Zn state it also emphasized that the introduction of FYM only” with 100% NPK contributed significantly to” the high level lead to its formation (Dwivedi and Dwivedi 2015) [7]. WSEX-Zn was recorded significantly increased and highest (0.63 mg kg⁻¹) and (0.26 mg kg⁻¹) in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. While, the lowest (0.24 mg kg⁻¹) and (0.05 mg kg⁻¹) WSEX-Zn content was recorded in control plot in both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. The WSEX-Zn found to be least among Zn fractions as Zn in this form is mobile and readily available for biological uptake in the environment. Similar results were found by Kumar and Babel (2011) and Ramzan et al.,, (2014) [15]. Similarly, Mohammed Nisab and Ghosh (2019) [5] revealed that the WSEX-Zn content was found to be least (1.0 percent) among the Zn fractions. Org-Zn in soil was obtained
significantly increased and highest (0.78 mg kg$^{-1}$) and (0.67 mg kg$^{-1}$) in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. While, the lowest (0.22 mg kg$^{-1}$) and (0.08 mg kg$^{-1}$) Org-Zn content was recorded in control plot in both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. This finding represented that organically bound fraction in soil system enhanced because of organic manure. It play a significant role in Zn availability. (Rathore et al., 1980 and El- Foulyet al., 2015)\cite{3}. AMOX-Zn soil was measured significantly increased and highest (0.89 mg kg$^{-1}$) and (0.58 mg kg$^{-1}$) in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. While, the lowest (0.52 mg kg$^{-1}$) and (0.10 mg kg$^{-1}$) AMOX-Zn content was recorded in control plot in both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Similar findings reported that may be characteristic to greater ability of amorphous sesquioxide to adsorb Zn because of their high specific surface characteristic to greater ability of amorphous sesquioxide to adsorb Zn because of their high specific surface area (Devis and Leckie, 1978)\cite{10}. Water logging may cause an increase in the amorphous forms of native soil (Mandal and Mandal, 1986)\cite{10}. Comparable results were also observed by Tehrani (2005)\cite{20}, Bahera et al., (2008)\cite{3} and Safari et al., (2009)\cite{16}. CRYOX-Zn in soil was recorded significantly increased and highest (1.86 mg kg$^{-1}$) and (0.43 mg kg$^{-1}$) in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. While, the lowest (0.48 mg kg$^{-1}$) and (0.20 mg kg$^{-1}$) CRYOX-Zn content was recorded in control plot in both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Among the non-residual fractions, the CRYOX-Zn fraction was found to be the major fraction of Zn and higher Zn concentrations of these stable fractions denote their importance as the storage fractions for soil Zn, although their solubility will determine how available they are for plant uptake. The results are quite near to the results observed by Singh et al., (1999)\cite{18} who reported that oxides bound Zn ranged from 2.05 to 3.40 mg kg$^{-1}$ in some rice growing red soils of India. The above results were also in conformity with the reports of Alvarez et al., (2001)\cite{1}, Bashir et al., (2007)\cite{2} and Chen et al., (2009)\cite{9}. This fraction was dominant when compared to water soluble plus exchangeable and organically bound zinc fractions due to predominance of crystalline iron oxide content. Similar results were also obtained by Wijebandara (2007)\cite{21}. The highest (74.84 mg kg$^{-1}$) and (59.79 mg kg$^{-1}$) residual Zn content was recorded in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. While, the lowest (59.84 mg kg$^{-1}$) and (44.91 mg kg$^{-1}$) residual Zn content was recorded in control plot in both the surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. Above findings indicated that the residual Zn was the dominant fraction among all the Zn fractions studied and is in agreement with the findings of Raja and Iyengar (1986)\cite{14} and Mishra et al., (2009)\cite{11}. Many researchers reported that large portion of Zn was in the residual fraction, with very little effect on the extraction plant uptake (Zang et al., 2014, and Kandali et al., 2016)\cite{22,9}. Wijebandara et al.,(2011)\cite{21}. The total variation in Zn may be because of soils being treated with variety of organic manures and inorganic fertilizers and thus affecting the availability of zinc in the soil thereby affecting the magnitude of the residual content of the soil. (Krishna kumar and Patty 1992). Similar results explained that the higher concentration of total zinc may be by reason of the increase in clay content as reported by Singh and Abrol (1986)\cite{19} and Sharma et al., (2002)\cite{17}. The available Zn content and its fractions in 100% NPK was considerably lower as compared to 100% NPK+Zn and 100% NPK+FYM.

<table>
<thead>
<tr>
<th>Soil(5 g)</th>
<th>1 M NH$_4$OAc at pH 7.0 (20 mL) shaken in a mechanical shaker for 1 h Centrifuge</th>
<th>Analyzed for Zn (Water soluble + Exchangeable Zn)</th>
<th>Supernatant Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue</td>
<td>20 mL 0.05 M Cu(OAc)$_2$ Shaken for 1 h, centrifuge</td>
<td>Supernatant</td>
<td>Analyzed for Zn (Organically bound Zn)</td>
</tr>
<tr>
<td>Residue</td>
<td>20 mL 0.2 M acidified NH$_4$(OX)$_2$ (pH 3.0) shaken for 1 h, centrifuge</td>
<td>Supernatant</td>
<td>Analyzed for Zn (Amorphous sesquioxide bound Zn)</td>
</tr>
<tr>
<td>Residue</td>
<td>40 mL 0.3 M Sodium Citrate 5 mL 1 M NaHCO$_3$ Stirred and kept on the waterbath at a temperature of 70-80 °C for 10 min + 1 g Na$_2$SO$_4$ Kept on waterbath 70-80 °C for 15 min With occasional stirring Centrifuge after cooling</td>
<td>Supernatant</td>
<td>Analyzed for Zn (Crystalline sesquioxide bound Zn)</td>
</tr>
<tr>
<td>Residue</td>
<td>5 mL conc. HNO$_3$ + 10 ml hydrofluoric acid + 10 ml perchloric acid</td>
<td>Supernatant</td>
<td>Analyzed for Zn (Residual Zn)</td>
</tr>
</tbody>
</table>

Fig 1: Sequential fractionation of zinc
Table 1: Effect of continuous application of fertilizer and organic manures on distribution of zinc fractions at surface and subsurface soils

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Available Zn</th>
<th>WSEX-Zn</th>
<th>Org-Zn</th>
<th>AMOX-Zn</th>
<th>CRYOX-Zn</th>
<th>Residual Zn</th>
<th>Total Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.67</td>
<td>0.50</td>
<td>0.05</td>
<td>0.22</td>
<td>0.08</td>
<td>0.10</td>
<td>0.48</td>
</tr>
<tr>
<td>50% NPK</td>
<td>0.67</td>
<td>0.50</td>
<td>0.24</td>
<td>0.08</td>
<td>0.52</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td>100% NPK</td>
<td>1.03</td>
<td>0.55</td>
<td>0.15</td>
<td>0.55</td>
<td>0.31</td>
<td>0.33</td>
<td>0.78</td>
</tr>
<tr>
<td>150% NPK</td>
<td>1.19</td>
<td>0.57</td>
<td>0.16</td>
<td>0.67</td>
<td>0.40</td>
<td>0.53</td>
<td>0.91</td>
</tr>
<tr>
<td>100% NPK+ZnSO4</td>
<td>1.94</td>
<td>0.94</td>
<td>0.25</td>
<td>0.78</td>
<td>0.89</td>
<td>0.58</td>
<td>1.86</td>
</tr>
<tr>
<td>100% NP</td>
<td>0.87</td>
<td>0.53</td>
<td>0.29</td>
<td>0.34</td>
<td>0.20</td>
<td>0.22</td>
<td>0.67</td>
</tr>
<tr>
<td>100% N+YM</td>
<td>0.76</td>
<td>0.52</td>
<td>0.28</td>
<td>0.52</td>
<td>0.15</td>
<td>0.19</td>
<td>0.63</td>
</tr>
<tr>
<td>100% NPK+GYS</td>
<td>1.43</td>
<td>0.62</td>
<td>0.18</td>
<td>0.54</td>
<td>0.57</td>
<td>0.48</td>
<td>0.98</td>
</tr>
<tr>
<td>50% NPK+BGA</td>
<td>0.69</td>
<td>0.49</td>
<td>0.25</td>
<td>0.44</td>
<td>0.11</td>
<td>0.13</td>
<td>0.81</td>
</tr>
<tr>
<td>50% NPK+GM</td>
<td>0.91</td>
<td>0.54</td>
<td>0.33</td>
<td>0.54</td>
<td>0.33</td>
<td>0.54</td>
<td>0.72</td>
</tr>
<tr>
<td>SEM (±)</td>
<td>0.12</td>
<td>0.08</td>
<td>0.03</td>
<td>0.32</td>
<td>0.06</td>
<td>0.07</td>
<td>0.19</td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td>0.35</td>
<td>0.15</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Conclusion
The continuous application of inorganic fertilizers and organic manures improved available zinc and its fractions in surface soil (0-15 cm). The highest available Zn content was recorded in 100% NPK+Zn in surface (0-15 cm) and subsurface (15-30 cm) soil, respectively. WSEX-Zn was found to be the least among the zinc fractions in both the depths. Among various forms of zinc fractions namely WSEX-Zn, Org-Zn, AMOX-Zn, CRYOX-Zn, residual Zn and total Zn, the residual zinc fraction contain highest amount of zinc compared to the other fractions of zinc under various treatments of LTFE. It is clearly indicated that various Zn fractions increased in surface soil layer with continuous application of Zn fertilizer. Similarly organic manure addition also increased the Zn content in the surface soil.

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