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Biogeoaccumulation of zinc in hybrid rice (*Oryza sativa* L.) in an Inceptisol amended with soil zinc application and its bioavailability to human being

Kiran Kumar Mohapatra [a,b], Satish Kumar Singh [a,*], Abhik Patra [a], Surendra Singh Jatav [a], Vishnu D. Rajput [c], Victoria Popova [d], Olesya Puzikova [d], Olga Nazarenko [e], Svetlana Sushkova [c]

[a] Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

[b] Department of Soil Science and Agricultural Chemistry, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

[c] Southern Federal University, Academy of Biology and Biotechnology, Rostov-on-Don, Russia

[d] Research Institute of Obstetrics and Pediatrics, Rostov-on-Don, Russia

[e] State Center of Agrochemical Service "Rostovsky", Rostov-on-Don, Russia

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Author(s)

K.K.Mohapatra

S.K.Singh *

A.Patra

S.S. Jatav

V.D.Rajput

V.Popova

O.Puzikova

O.Nazarenko

S.Sushkova

* Corresponding author

Abstract

Soil Zn amended is an efficient agronomical Zn biofortification approach in rice. However, it is still need to know if higher rate of Zn over recommended dose can influence other essential nutrient uptake, high accumulation of Zn in soils and health risk for human consumption. This study was conducted by taking ten treatments (T_1: control, T_2: RDF, T_3: RDF + 1.25 mg kg^{-1}, T_4: RDF + 2.5 mg kg^{-1}, T_5: RDF + 3.75 mg kg^{-1}, T_6: RDF + 5 mg kg^{-1}, T_7: RDF + 6.25 mg kg^{-1}, T_8: RDF + 7.5 mg kg^{-1}, T_9: RDF + 8.75 mg kg^{-1}, T_{10}: RDF + 10 mg kg^{-1}) on hybrid rice in Zn (1.20 mg kg^{-1}) enriched soil. The findings have shown that 6.25 mg kg^{-1} Zn application significantly increased crop growth and grain concentrations of N, K, Zn, Cu and Fe by 71.4, 125, 78.9, 28.5 and 2.4%, respectively. Nutrient harvest index was significantly affected by ranged between 29.1–36.4%. Application of Zn at 6.25 mg kg^{-1} (T_7) recorded the highest Zn concentration in grain (28.2 mg kg^{-1}) and bioavailability of the fortified Zn (2.05 mg Zn day^{-1}). The lowest phytatic acid concentration in grain was recorded in T_8 (RDF + Zn at 7.5 mg kg^{-1}) and after that a significant increase was observed. Transfer coefficient was inversely behaving with Zn application and ranged between 6.03–18.0 grain. The average daily intake of Zn was ranged between 0.075–0.118 mg^{-1} kg^{-1} day. Across different treatments the Zn build-up factor, geo-accumulation index and soil enrichment factor was ranged between 0.98–4.90, -0.61–1.70 and 0.24–1.82, respectively in post-harvest soil. In conclusion, agronomic biofortification of Zn through soil applications at 6.25 mg Zn kg^{-1} was a sustainable way to improving growth and grain Zn, N, K, Cu and Fe uptake of hybrid rice to meet human recruitment.

Keywords: Nutrient harvest index, rice, zinc, zinc balance sheet, Zn build-up factor.

Introduction

The most frequent nutritional problems, particularly in developing nations, are zinc (Zn), vitamin A, and iron (Fe) deficiencies (Welch and Graham, 2005). It was estimated that 17.3% of people of worldwide, and 500,000 children die due to nutritional deficiency of Zn (Wessells and Brown, 2012). Mostly pregnant

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woman and children are affected by Zn deficiency (Jatav et al., 2019). In human, Zn plays vital role in many important catalytic, structural, and regulatory functions like enzymes catalytic activity that are involved in DNA replication, cell division, energy metabolism, growth, structure stability of protein, regulation of antioxidants, leptin and insulin signaling. Moreover, Zn deficiency in human causes malfunctioning of the immunity system, male hypogonadism, appetite loss, skin lesions, poor wound healing, diarrhea, delayed sexual maturity, slower growth rate (Prasad, 2013). In plants, Zn plays a key role in the proper functioning of different biochemical pathways by activating a broad range of enzymes and proteins. These are primarily concerned with carbohydrate, auxin and amino acid metabolism, reactive oxygen species (ROS) detoxification, pollen formation, the integrity of cellular membranes, and disease resistance (Hafeez et al., 2013). However, in a deficient Zn situation, crop yields may be decreased by 20% without any noticeable symptoms (Cakmak, 2000; Broadley et al., 2007).

Zinc bioavailability in soil affects plant uptake of Zn and also human and animal nutrition of Zn. The deficiency of Zn affects almost one third of the global population (Hotz and Brown, 2004) and is the fifth leading cause of human mortality in developing countries (Cakmak and Kutman, 2018). In early life stages, Zn deficiency may influence embryogenesis, hypogonadism, increased vulnerability to serious diseases, and decreased mental development. There is no Zn reservoir in the human body, therefore, the bioavailability of Zn through food or supplements must be regularly provided in order to avoid its deficiency. Nutrient fortification is a preferred way of addressing the problem of the undernourished rural population. However, majority of rural populations are unable to obtain a variety of diets, supplements and commercially fortified foods. Bio-fortification is a method of enhancing the bio-availability of vital elements in edible crop portions through agronomic action or genetic selection (White and Broadly, 2011).

Wheat and rice are major staple food crops of India which constitute about 60–70% of daily calorie uptake. The rice grain is very low in Zn and contains anti-nutrition compounds like phytates which reduced bioavaility of Zn (Kumar et al., 2017). Cereals normally comprise low Zn *i.e.,* 15–30 mg Zn kg^{-1} compared to a sufficient 40–60 mg Zn kg^{-1} concentration for better nutrition but, preferred over others being staple food of a large population. Zinc bio-fortification of rice could save lives in most populous countries like India and China between 1.6–2.3 million DALYs (disability-adjusted life year) and 0.4–1.5 million DALYs (De Steur et al., 2012) per year, respectively. Genetic bio-fortification of food crops faces several challenges and takes many years to get beneficial effects. Agronomic bio-fortification is, therefore, a feasible approach for developing nations, based on micronutrient-dense cultivar exploitation (Sharma et al., 2017). Zinc should be applied as seed coating, soil application or, foliar spray at higher quantity for fortification of crop with Zn and for better translocation of Zn to grain from soil (Singh and Prasad, 2014). Das et al. (2018) noted that Zn application will not only correct crop Zn deficiency and enhance crop yield and productivity, but also help to increase Zn concentration and reduce anti-nutrient (phytate) concentration in grain.

Rice is the second largest staple food in the world and cultivated in more than 100 countries. India is the world's largest producer and fourth largest exporter of rice (FAO, 2017). Generally, after polishing white rice contain low quantities of Zn (11–16 µg g^{-1}), thus consumption of rice fails to satisfy the estimated average requirement (EAR) of Zn in human being (Mayer et al., 2011). It was reported that increase of 12 µg Zn g^{-1} of milled rice could provide at least 25% of the Zn EAR for pre-school children (Alloway, 2009). Zn nutritional allowance for babies is 3–5 mg day^{-1}, while for kids 1–10 years of age it is 10 mg day-1, for males 12 mg day^{-1} and for lactating females 16–19 mg day^{-1} (Alloway, 2009). It is well documented that agronomic bio-fortification of cereals is one of feasible way to eradicate this Zn malnutrition problem (Cakmak 2008; Cakmak and Kutman, 2018). Zn fertilizer application in soil increases the Zn build-up in soil and food grain but causes other nutrient imbalances due to antagonistic interaction among the micronutrient cations (Jiao et al. 2012).

Excessive Zn buildup in soil promotes crop uptake, resulting in an unexpectedly high Zn concentration in grain (Noulas et al., 2018; Wongsasuluk et al., 2018). In contrast to higher Zn concentration in soil can affect crop absorption of other micronutrients. (Kolašinac et al., 2018). Our hypothesis is that different levels of Zn fertilizer application, especially at higher rate than soil test value in hybrid rice would increase the uptake of Zn by plant, and thus, represents a better Zn nutritional security. The precise objectives of this study were: (i) to evaluate the different levels of Zn soil application on uptake of major and micronutrient in rice, (ii) to assess the Zn balance sheet, Zn accumulation indices in soil-plant system and average daily intake of Zn through consumption of rice grain that was came from different level of Zn applications. As a result, this study will lead to a better understanding of Zn bio-fortification in paddy in relation to various zinc-soil-plant indices.

Material and Methods

Study area

A pot experiment was conducted during the *kharif* (July – November) seasons of 2018–19 at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (25^{0}26'N, 82^{0}99' E, 80.7 m above sea level). The climate is semi-arid to sub-humid, hot summers and simply pleasant cold winters. The mean annual rainfall is 1100 mm. The meteorological observation recorded during rice growth period 2018–19 (*kharif* season) was presented in Figure 1. The experimental soil was sandy clay loam in texture with 58.3% sand, 25.7% silt and 16.0% clay. The soil had pH 7.71, EC 0.13 dS m^{-1}, soil organic carbon (SOC) 8.01 g kg^{-1} and DTPA-extractable Zn 1.20 mg kg^{-1} (Table 1).

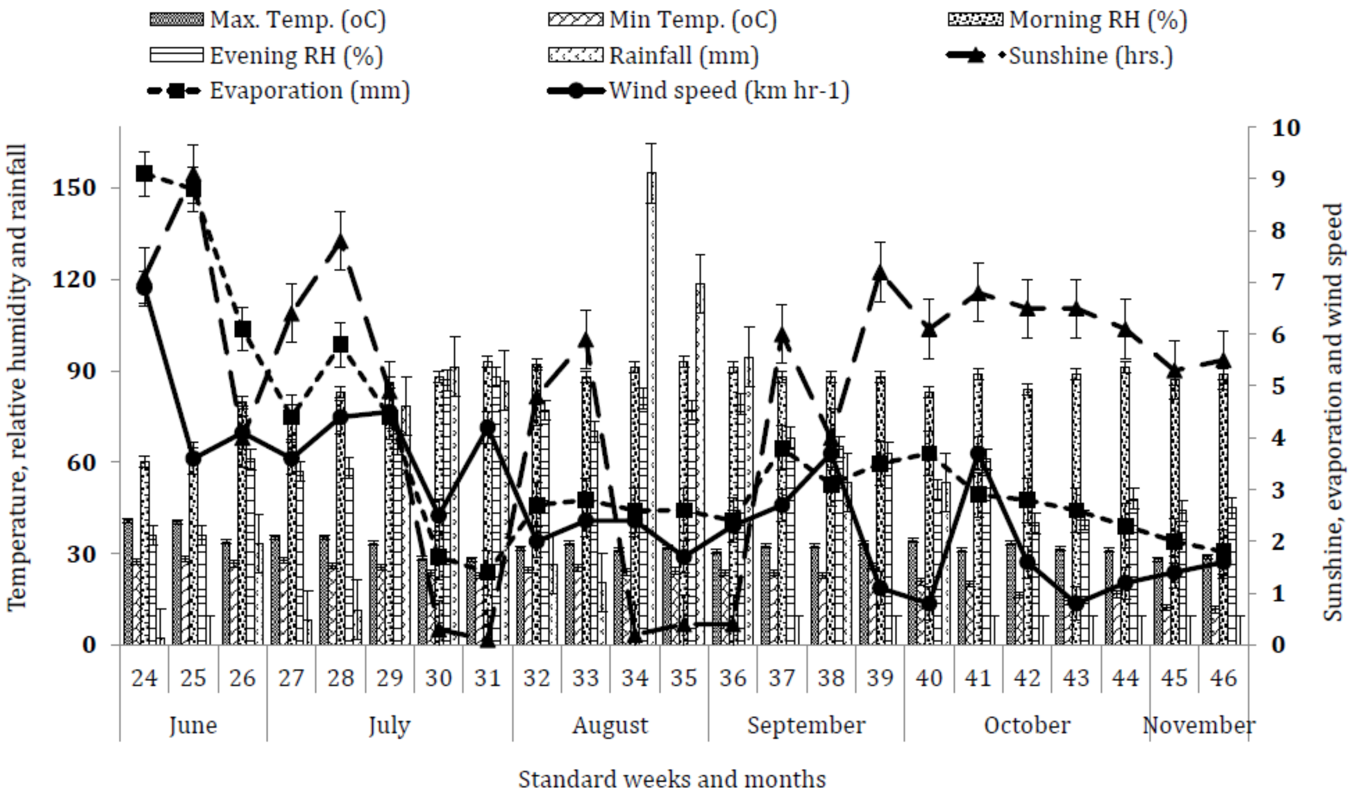

Figure 1. Meteorological data during rice growing period (June to November, 2018)
Error bars identifies standard error of mean of different treatments

Experimental setup

The completely randomized experimental design (CRD) employed with ten treatments *i.e.,* T_1: control (no fertilizer), T_2: recommended dose of fertilizer (RDF), T_3: RDF + Zn @ 1.25 mg kg^{-1}, T_4: RDF + Zn @ 2.5 mg kg^{-1}, T_5: RDF + Zn @ 3.75 mg kg^{-1}, T_6: RDF + Zn @ 5 mg kg^{-1}, T_7: RDF + Zn @ 6.25 mg kg^{-1}, T_8: RDF + Zn @ 7.5 mg kg^{-1}, T_9: RDF + Zn @ 8.75 mg kg^{-1}, T_{10}: RDF + Zn @ 10 mg kg^{-1} with three replications and Arize® H 6444 (hybrid rice) was taken as test crop. Standard practices were followed for the cultivation of rice and it was harvested in the second half of October in both study years. State (Utter Pradesh) recommended dose of fertilizer (N, P and K) for hybrid rice was 150, 60 and 60 kg ha^{-1}, respectively. Urea, di-ammonium phosphate and muriate of potash was the source of RDF and 50% N and 100% of P and K was applied in solution form before transplanting of rice and remaining 50% of N was applied in two equal splits at 30 and 60 days after transplanting. Zinc fertilization was done as soil application through zinc sulphate ($ZnSO_4.7H_2O$) in solution form.

Table 1. Initial properties of experimental soil

Parameters	Values
pH (1:2.5; soil:water)	7.71
EC (dS m^{-1})	0.13
Organic carbon (g kg^{-1})	8.01
N (mg kg^{-1})	138
P (mg kg^{-1})	11.0
K (mg kg^{-1})	89.5
S (mg kg^{-1})	14.6
Zn (mg kg^{-1})	1.20
Fe (mg kg^{-1})	45.2
Mn (mg kg^{-1})	13.2
Cu (mg kg^{-1})	5.20
B (mg kg^{-1})	0.29
Sand (%)	58.3
Silt (%)	25.7
Clay (%)	16.0
Texture	Sandy clay loam

Plant and soil analysis

Different growth attributing characteristics like plant height, number of tillers, greenness index (SPAD value) were recorded at 30, 60 and 90 days after transplanting. The yield attributing characters were measured at maturity *viz.,* average number of panicles per pot, number of grains per panicle and panicle length. Rice was harvested at 120 days after transplanting. Then plant samples were washed in 0.2% liquid detergent solution followed by 0.1 N hydrochloric acid (HCl) solution and de-ionized water. The plant samples were kept in hot air oven at 70 °C till the constant weight was gain. The grain yield, straw yield and 1000 grain weight per pot was recorded. Initial and post-harvest soil samples were analyzed for mechanical analysis of soil by using international pipet method (Piper, 1966), pH and electrical conductivity (EC) by (Sparks, 1996), organic carbon by method of Walkley and Black (1934), DTPA-extractable Zn, Cu, Mn and Fe (Lindsay and Norwell, 1978) by AAS (Agilent FS 240, 2019). The plant samples (grain and straw) were wet digested in a di-acid mixture and analyzed for P, K, Fe, Cu, Mn and Zn using AAS (Agilent FS 240, 2019) by Tandon (2001).

Nutrient uptake

$$\text{Nutrient uptake (mg pot}^{-1}) = \frac{N_C \times Y}{1000}$$

Where, Nc is nutrient content (mg kg^{-1}) and Y is biomass (g pot^{-1}).

Nutrient harvest index

Uptake of a particular nutrient is the product of the grain yield and concentration of respective nutrient in grain. Total nutrient uptake is the sum of nutrient uptake of grain and straw.

Nutrient harvest index (NHI) of different nutrients was calculated using Das et al. (2010) equation:

$$\text{NHI (\%)} = \frac{\text{Uptake of particular nutrient by grain kg/ha}}{\text{Total uptake of that nutrient in biomass kg/ha}}$$

The transfer coefficient (TC), the enrichment factor (EF), and the geo-accumulation index (Igeo) were calculated in order to assess the degree of Zn enrichment in soil and the plant's ability to accumulate Zn from soils and translocate it from roots to grain.

Estimation of phytate concentration and bioavailability of Zn

The technique outlined by Dai et al. (2007) was used to determined phytic acid from the rice sample. Miller et al. (2007) provided a trivariate model of Zn absorption for quantitative assessment of Zn bioavailability.

$$\text{TAZ} = 0.5 \times \left\{ \left(A_{MAX} + TDZ + \left(1 + \frac{TDP}{K_P}\right) \right) - \sqrt{\left(AMAX + TDZ + \left(1 + \frac{TDP}{K_P}\right) \right)^2 - (4A_{MAX} \times TDZ)} \right\}$$

Where, Maximum absorption (A_{MAX}) = 0.091, Equilibrium dissociation constant of Zn–receptor binding reaction (K_R) = 0.680 and Equilibrium dissociation constant of Zn–phytate binding reaction (K_P) =0.033, respectively. These values were calculated based on Zn homeostasis in human intestine. The total daily-absorbed Zn (TAZ) (mg Zn day^{-1}) is a function of total daily dietary phytate (TDP) (mmol phytate day^{-1}) and total daily dietary Zn (TDZ) (mmol Zn day^{-1}). Here, TAZ value was calculated based on an adult human's average daily intake of 300 g of rice grain.

Transfer coefficient (TC)

The transfer coefficient (TC) of Zn is the ratio of the Zn concentration in the plant with respect to Zn concentration in soil (mg kg^{-1}) (Adamczyk-Szabela et al., 2017). It explains the ability of the plant to accumulate Zn with respect to its soil concentration.

The plant enrichment factor (PEF)

Plant enrichment factor (PEF) is used to evaluate the levels of Zn concentration and accumulation in plants growing on Zn treated soil to plants growing on control soil (Kisku et al., 2000). It's the ratio of Zn concentration in Zn-treated soil/plant to Zn concentration in control condition. PEF values larger than 1 indicate increased Zn availability and distribution in Zn treated soil is in higher compared to their reference values (control).

Zinc balance sheet

Balance sheet of Zn in soil was determined by using the formula (Bera and Ghosh, 2013).

$$B_{Zn} = Y_{Zn} - (X\text{-}A) - Zn$$

Where B = Balance sheet of nutrient, Y_{Zn} = Zn Uptake by crop, X= Zn concentration in Initial the soil, A = Zn concentration in post-harvest soil, Zn = Zn added through fertilizer.

Average daily intake

The average daily intake (ADI) of Zn was calculated using standard formula (Khan et al., 2008).

$$ADI = \frac{C_{Zn} \times Dfood\ intake}{average\ BW}$$

where C_{Zn} is the Zn concentration in polish white rice, Dfood intake is the average daily rice intake (0.300 kg person^{-1} day^{-1}) and BWaverage weight represent body weight of the individuals (70 kg for adults) (Khan et al., 2010; GFBIC, 2014; Doabi et al., 2018).

Zinc build up factor

Zinc build up factor is the ratio obtained by dividing the concentration of Zn in soil by their background values (control):

BF = CS/CRefS

where CS is Zn concentrations in Zn treated soil and CRefS is background Zn concentration in control soil (Shaheen et al., 2017).

The index of geo-accumulation (Igeo)

The index of geo-accumulation (Igeo) which also assesses the Zn potential in soil is calculated as

$$Igeo = \log2(C_{Zn} / 1.5Bn)$$

where C_{Zn} is Zn concentration in the Zn fertilizer treated soil and Bn is the geochemical background concentration of the Zn (Antoniadis et al., 2017).

Soil Enrichment factor

$$SEF = (ZnS/ FeS) / (ZnRefS / FeRefS)$$

Where *Zn*S is the Zn concertation in Zn treated soil, whereas FeS is the Fe concentration on their respective treatment. Similarly, ZnrefS and FeRefS is the Zn and Fe concentration in control pot.

The level of enrichment of metal is then classified as EF < 2 (marked as "deficient enrichment"), EF=2–5 ("moderate enrichment"), EF=5–20 ("significant enrichment"), EF=20–40 ("very high enrichment"), and EF > 40 ("extremely high enrichment") (Liu et al., 2005).

Statistical analysis

Using SPSS version 16.0 software, the data were statistically analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to see if the difference between the treatments was significant at $p \leq 0.05$.

Results and Discussion

Uptake of macro- and micro-nutrients

Based on the result of different level of Zn fertilization had a significant effect on uptake of macronutrient (Table 2). The uptake of N, P and K by hybrid rice grain ranged from 0.18–0.60, 0.04–0.11 and 0.04–0.10 g pot^{-1}, respectively. While macronutrient (N, P and K) uptake in straw varied from 0.11 to 0.28, 0.06–0.09 and 0.37 to 0.99 g pot^{-1} respectively. Significantly higher total nitrogen (N) uptake was recorded in 6.25 mg Zn kg^{-1} (T_7) (67.9 % increase over RDF). Similarly, higher total K uptake was recorded at 6.25 mg Zn kg^{-1} (T_7) (61.2 % over RDF), While higher total P uptake by hybrid rice was recorded at 2.5 mg Zn kg^{-1} T_4 (5% over RDF). All Zn treatments, 6.25 mg Zn kg^{-1} (T_7) were recorded most efficient in total macronutrient uptake by hybrid rice. The Zn uptake by grain, straw and total uptake varied between 0.34–1.02, 0.83–2.05 and 1.18–3.06 mg pot^{-1}, respectively (Table 3). The treatment received T_5, T_9, and T_{10} were statistically at per with each other. The maximum total Zn uptake was recorded in T_7, which 58.5% higher over RDF.

Table 2. Effect of zinc application on macronutrients uptake (g pot^{-1}) in various plant parts of rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	N (g pot^{-1})		P (g pot^{-1})		K (g pot^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw
T_1 (Control)	0.18^{g}	0.11^{g}	0.04^{f}	0.06^{c}	0.04^{f}	0.37^{h}
T_2 (RDF*)	0.35^{ef}	0.18^{f}	0.09^{bc}	0.10^{a}	0.06^{e}	0.55^{f}
T_3 (RDF + $Zn_{1.25}$)	0.38^{de}	0.20^{e}	0.10^{ab}	0.09^{a}	0.07^{de}	0.58^{ef}
T_4 (RDF + $Zn_{2.5}$)	0.44^{c}	0.22^{cd}	0.11^{a}	0.09^{a}	0.08^{c}	0.61^{de}
T_5 (RDF + $Zn_{3.75}$)	0.48^{b}	0.26^{c}	0.10^{ab}	0.09^{a}	0.08^{b}	0.70^{bc}
T_6 (RDF + $Zn_{5.0}$)	0.55^{b}	0.26^{b}	0.09^{bc}	0.08^{b}	0.10^{a}	0.71^{b}
T_7 (RDF + $Zn_{6.25}$)	0.60^{a}	0.28^{a}	0.09^{bc}	0.08^{b}	0.09^{a}	0.91^{a}
T_8 (RDF + $Zn_{7.5}$)	0.46^{cd}	0.22^{cd}	0.07^{e}	0.07^{bc}	0.07^{d}	0.64^{cd}
T_9 (RDF + $Zn_{8.75}$)	0.44^{c}	0.17^{f}	0.08^{d}	0.06^{c}	0.07^{d}	0.47^{g}
T_{10} (RDF + $Zn_{10.0}$)	0.41^{c}	0.17^{f}	0.09^{bc}	0.08^{b}	0.07^{d}	0.47^{g}
SEm ±	0.01	0.01	0.003	0.003	0.002	0.02
CD ($p \leq 0.05$)	0.03	0.02	0.01	0.01	0.005	0.06

*Recommended dose of fertilizer

While total uptake of Cu, Fe, Mn and B varied between 0.18–0.44, 6.49–12.8, 4.92–10.1 and 0.65–1.27 mg pot^{-1} (Table 3). Significantly higher grain and straw uptake of Cu, Fe, Mn and B was recorded in T_6 (21 mg pot^{-1}) and T_4 (2.67 mg pot^{-1}), T_4 (1.95 mg pot^{-1}) and T_3 (11.0 mg pot^{-1}), T_3 (0.76 mg pot^{-1}) and T_5 (9.46 2.67 mg pot^{-1}), T_5 (2.67 mg pot^{-1}) and T_4 (2.67 mg pot^{-1}). Above 6.25 mg kg^{-1} of Zn soil application, total uptake of Cu, Fe and B was significantly decreased over RDF. Among all treatment T_4 was recorded most efficient in micronutrient (except Zn) uptake by hybrid rice. Nutrient uptake being functions of dry matter production and partly due to increase in nutrient concentration. The highest total uptake of nutrients (N, K, Zn, Fe and Mn) was recorded at 6.25 mg kg^{-1} and the lowest uptake at control.

Table 3. Effect of zinc application on micronutrients uptake (g pot^{-1}) in various plant parts of rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	Zn (mg pot^{-1})		Cu (mg pot^{-1})		Fe (mg pot^{-1})		Mn (mg pot^{-1})		B (mg pot^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
T_1 (Control)	0.34^{h}	0.83^{f}	0.09^{c}	0.09^{e}	0.72^{f}	5.77^{d}	0.28^{e}	4.64^{e}	0.29^{c}	0.36^{c}
T_2 (RDF*)	0.57^{g}	1.38^{de}	0.14^{c}	0.18^{dc}	1.64^{c}	10.4^{ab}	0.56^{c}	8.27^{bc}	0.50^{ab}	0.55^{ab}
T_3 (RDF + $Zn_{1.25}$)	0.63^{fg}	1.52^{cd}	0.18^{a}	0.26^{a}	1.79^{abc}	11.0^{a}	0.73^{a}	8.92^{ab}	0.50^{ab}	0.64^{a}
T_4 (RDF + $Zn_{2.5}$)	0.72^{de}	1.65^{c}	0.20^{a}	0.24^{ab}	1.97^{a}	10.3^{ab}	0.67^{ab}	8.87^{ab}	0.53^{a}	0.73^{a}
T_5 (RDF + $Zn_{3.75}$)	0.79^{cd}	1.87^{b}	0.20^{a}	0.22^{b}	1.84^{ab}	10.6^{a}	0.63^{bc}	9.46^{a}	0.57^{a}	0.59^{ab}
T_6 (RDF + $Zn_{5.0}$)	0.92^{b}	1.87^{b}	0.21^{a}	0.21^{bc}	1.68^{bc}	10.2^{ab}	0.59^{bc}	7.98^{bc}	0.53^{a}	0.67^{a}
T_7 (RDF + $Zn_{6.25}$)	1.02^{a}	2.05^{a}	0.18^{a}	0.19^{c}	1.63^{c}	10.9^{a}	0.64^{bc}	9.38^{a}	0.53^{a}	0.54^{ab}
T_8 (RDF + $Zn_{7.5}$)	0.84^{c}	1.64^{c}	0.15^{b}	0.16^{d}	1.40^{d}	9.61^{b}	0.48^{cd}	7.52^{c}	0.41^{b}	0.48^{bc}
T_9 (RDF + $Zn_{8.75}$)	0.74^{de}	1.29^{e}	0.15^{b}	0.11^{e}	1.22^{de}	7.67^{c}	0.47^{cd}	5.53^{cd}	0.42^{b}	0.39^{bc}
T_{10} (RDF + $Zn_{10.0}$)	0.67^{ef}	1.25^{e}	0.15^{b}	0.12^{e}	1.16^{e}	7.34^{c}	0.44^{d}	5.56^{cd}	0.43^{b}	0.41^{bc}
SEm ±	0.02	0.05	0.01	0.01	0.06	0.31	0.03	0.29	0.02	0.02
CD ($p \leq 0.05$)	0.07	0.15	0.02	0.02	0.17	0.92	0.08	0.87	0.05	0.06

*Recommended dose of fertilizer

Results might be because of increased nutrient concentrations in rice grain and straw, as well as increased yield of rice grain and straw. It has been noted that optimal Zn levels can improve rice nutrients uptake; our results agreed with previous research (Li et al., 2007; Dash et al., 2010; Rutkowska et al., 2014; Xue et al., 2014). The synergistic impact of Zn and N is mostly related to increased Zn availability in soil because N main responsible for soil acidity (Pooniya et al., 2018). Zinc fertilizer also had a significant impact on P absorption in rice grain and straw. This could be related to an increase in N levels in plants caused by $ZnSO_4.7H_2O$, as well as higher Basmati rice yields, which resulted in enhanced total P uptake. These results are in conformity with the findings of Pooniya and Shivay, (2013); Shivay et al. (2015). It might be due to antagonistic effect of P with Zn. Gohil et al. (2017) reported that in case of P uptake, lower rate Zn application recorded significantly more uptake of P. However, their application at higher levels significantly reduced the P uptake by both grain and straw portion of rice. Gohil et al. (2017) reported that Mn and Cu uptake was significantly affected by Zn fertilization.

Nutrient harvest index

Nutrient harvest index was calculated by taking nutrient uptake of particular nutrient. Table 4 revealed that different level of Zn soil application had a significant effect on nutrient harvest index of different nutrient. Results indicated that among different treatments; application of RDF + Zn 8.75 mg kg^{-1} in to soil has higher macro and micronutrient harvest index compared to other treatments. Among macronutrient (N, P and K), NHI value of N (72.5%) is greater than P (55.1%) followed by K (12. 4%). Where as in micronutrients, NHI value of Cu is higher than B followed by Zn, Fe and Mn. The values of nutrients nitrogen, phosphorus, potassium, iron, zinc, copper, manganese, and boron (N, P, K, Fe, Zn, Cu, Mn, and B) harvest index were greater under 7.5 mg kg^{-1} Zn application treatments (Table 4). The higher nutrient harvest index with respect to control could be attributable to the fact that under sustainable nutrient supply conditions, the plant seeks to take more from the soil and converts the most towards seeds in order to complete the life-cycle. Dass et al. (2010) reported the nutrient harvest index of N, P and K in rice. Kumar et al. (2015) reported the Nutrient harvest index concept in okra.

Table 4. Effect of zinc application on nutrient harvest index (%) of rice (Different letters for each parameter show significant difference at $p \le 0.05$ by Duncan's Multiple Range Test)

Treatment	N (%)	P (%)	K (%)	Zn (%)	Cu (%)	Fe (%)	Mn (%)	B (%)
T_1 (Control)	61.8^{d}	37.7^{c}	8.52^{f}	29.5^{d}	51.0^{bc}	11.1^{c}	5.73^{d}	44.4^{cde}
T_2 (RDF*)	65.8^{c}	49.9^{bc}	10.4^{cde}	29.2^{d}	43.2^{de}	13.6^{b}	6.40^{b}	47.7^{abcd}
T_3 (RDF + $Zn_{1.25}$)	65.8^{c}	52.0^{ab}	10.6^{cd}	29.1^{d}	41.2^{e}	13.9^{b}	7.60^{ab}	44.0^{de}
T_4 (RDF + $Zn_{2.5}$)	65.8^{c}	54.7^{b}	11.3^{abc}	30.5^{cd}	46.4^{cde}	15.9^{a}	7.07^{abc}	42.1^{e}
T_5 (RDF + $Zn_{3.75}$)	64.9^{c}	51.0^{abc}	10.8^{bcd}	29.8^{d}	47.4^{cde}	14.8^{ab}	6.22^{cd}	49.1^{abc}
T_6 (RDF + $Zn_{5.0}$)	68.1^{bc}	52.1^{ab}	11.9^{ab}	33.0^{bc}	50.1^{bc}	14.2^{ab}	6.84^{abcd}	44.1^{de}
T_7 (RDF + $Zn_{6.25}$)	68.1^{bc}	52.4^{ab}	9.41^{ef}	33.2^{bc}	48.6^{cd}	12.9^{bc}	6.37^{bcd}	49.6^{ab}
T_8 (RDF + $Zn_{7.5}$)	67.8^{bc}	46.9^{b}	9.81^{de}	33.8^{ab}	47.8^{cd}	12.7^{bc}	6.01^{cd}	46.0^{bcde}
T_9 (RDF + $Zn_{8.75}$)	72.5^{a}	55.1^{a}	12.4^{a}	36.4^{a}	58.1^{a}	13.8^{b}	7.98a	51.6^{a}
T_{10} (RDF + $Zn_{10.0}$)	70.8^{ab}	52.3^{ab}	12.1^{a}	35.1^{ab}	55.2^{ab}	13.7^{b}	7.29^{abc}	50.9^{a}
SEm ±	0.99	1.33	0.37	0.90	5.76	0.64	0.39	4.30
CD ($p \le 0.05$)	2.92	3.91	1.08	2.66	1.95	1.88	1.16	1.46

*Recommended dose of fertilizer

Zn balance sheet

Zinc balance sheet were calculated and presented in the Table 5. In all the treatments, except for control and RDF, Zn balances were negative. The results of our study showed that The Apparent Zn balance was highly negative at higher doses of Zn festination. The higher negative value of nutrient balance was recorded at T_{10} and least value was at control. Additional application of Zn influences the soil properties in various ways. So, Zn exceeds the amount of higher negative balance.

The higher negative Zn balance recorded under the treatment where higher amount of Zn fertilization applied. It is one of the important micronutrients available to plants through diffusion and mass flow in the soil environment. The availability of Zn, as well as its uptake and utilization by rice, are thus intimately tied to productivity, but are influenced by a variety of abiotic and biotic factors in the soil-plant system, such as cultivar, nutrient input, soil and climatic condition. This might be due to the fact that higher amount of Zn application helps in buildup Zn concentration in soil and plant. Similar type of result was recorded by Bera and Ghosh (2013).

Table 5. Effect of zinc application on zinc balance sheet in rice (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatment	Zn input (mg pot^{-1}) [A]			Zn output (mg pot^{-1}) [B]			Apparent Zn balance (mg pot^{-1})
	Initial soil Zn [a]	Soil Zn applied [b]	Total Zn in soil [a + b]	Total Zn uptake [c]	Soil Zn after harvest [d]	Total Zn output [c + d]	[A - B]
T_1 (Control)	12.0	0	12.0	1.18^{f}	11.7^{f}	12.9^{f}	0.94^{a}
T_2 (RDF*)	12.0	0	12.0	1.95^{e}	10.7^{f}	12.7^{f}	0.67^{a}
T_3 (RDF + $Zn_{1.25}$)	12.0	11.1	23.1	2.15^{d}	15.8^{ef}	18.0^{ef}	-5.10^{b}
T_4 (RDF + $Zn_{2.5}$)	12.0	22.3	34.3	2.37^{c}	19.6^{def}	21.9^{de}	-12.3^{c}
T_5 (RDF + $Zn_{3.75}$)	12.0	33.4	45.4	2.67^{b}	24.3^{cde}	27.0^{d}	-18.4^{d}
T_6 (RDF + $Zn_{5.0}$)	12.0	44.6	56.6	2.79^{b}	26.4^{cd}	29.2^{cd}	-27.4^{e}
T_7 (RDF + $Zn_{6.25}$)	12.0	55.8	67.8	3.06^{a}	32.9^{c}	36.0^{c}	-31.8^{fg}
T_8 (RDF + $Zn_{7.5}$)	12.0	66.6	78.6	2.48^{c}	46.9^{b}	49.4^{b}	-29.2^{ef}
T_9 (RDF + $Zn_{8.75}$)	12.0	78.1	90.1	2.03^{de}	53.1^{ab}	55.2^{ab}	-34.9^{g}
T_{10} (RDF + $Zn_{10.0}$)	12.0	89.3	101.3	1.92^{e}	58.7^{a}	60.6^{a}	-40.7^{h}
SEm ±	-	-	-	0.06	2.82	2.81	1.09
CD ($p \leq 0.05$)	-	-	-	0.19	8.34	8.30	3.24

*Recommended dose of fertilizer

Phytate concentration and Zn bioavailability

The maximum reduction in phytate concentration was recorded in the treatment receiving soil application of 7.5 mg kg^{-1} (T_8) which is 27% lower than RDF (T_2) (Table 6) and a significant increase was noticed at higher dosed of Zn application (T_9 and T_{10}). It may be due to the dilution effect caused with increase in yield by application of Zn fertilizers. However, at higher levels of Zn application (T_9 and T_{10}), the phytate concentration increased significantly in grain, this might be due to the reduction in grain yield of rice. The range of phytate: zinc molar ratio was ranges from 20–41.2 (Table 6). The lowest phytate: zinc molar ratio was noticed in soil when 6.25 mg Zn kg^{-1} was applied in soil (T_7). The phytate: zinc molar is indicative of higher Zn availability with corresponding decrease in phytate: Zn molar ratio. The decrease in phytate: the zinc molar ratio was not only dependent on the decrease in the concentration of phytic acid (PA), but also due to the greater concentration of Zn in rice grains. Various levels of Zn implementation significantly impacted total absorbed zinc (TAZ) in rice grain (Table 6). Its concentration increased progressively with application of Zn in soil. The maximum TAZ was observed in treatment receiving 6.25 mg Zn kg^{-1} (T_7) *i.e.,* 2.05 mg Zn day^{-1} and the minimum was in RDF (T_2). The TAZ significantly increased with increasing doses of Zn application up to 7.5 mg Zn kg^{-1} and then significantly reduced.

Table 6. Effect of zinc application on phytate, Zn, phytate: Zn molar ratios and total absorbed zinc (TAZ) in rice grain (Different letters for each parameter show significant difference at $p \leq 0.05$ by Duncan's Multiple Range Test)

Treatments	Phytate (mg kg^{-1})	Zn (mg kg^{-1})	Phytate: Zn molar ratios	TAZ (mg Zn day^{-1})
T_1 (Control)	7260^{d}	17.5^{f}	41.2^{f}	1.27^{e}
T_2 (RDF*)	6177^{b}	19.5^{ef}	31.4^{e}	1.49^{d}
T_3 (RDF + $Zn_{1.25}$)	5930^{ab}	21.3^{de}	27.6^{cd}	1.62^{bc}
T_4 (RDF + $Zn_{2.5}$)	5922^{ab}	21.9^{d}	26.8^{cd}	1.66^{bc}
T_5 (RDF + $Zn_{3.75}$)	5769^{a}	23.5^{cd}	24.3^{bc}	1.77^{b}
T_6 (RDF + $Zn_{5.0}$)	5706^{a}	26.0^{ab}	21.7^{ab}	1.93^{a}
T_7 (RDF + $Zn_{6.25}$)	5671^{a}	28.2^{a}	20.0^{a}	2.05^{a}
T_8 (RDF + $Zn_{17.5}$	5649^{a}	27.6^{a}	20.3^{a}	2.02^{a}
T_9 (RDF + $Zn_{8.75}$)	6788^{c}	25.2^{bc}	26.8^{cd}	1.75^{bc}
T_{10} (RDF + $Zn_{10.0}$)	6829^{c}	23.2^{cd}	29.2^{de}	1.64^{c}
SEm ±	112	0.77	1.06	0.05
CD ($p \leq 0.05$)	332	2.26	3.12	0.14

*Recommended dose of fertilizer

According to Wei et al. (2012), Zn treatment increases Zn concentration and decreases phytic acid, resulting in higher bioavailable Zn accumulation in rice grains. Wang et al. (2021) also revealed that phytate content in rice grain decreases as the dose of the application increases. Yaseen and Hussain (2021) and Akram et al. (2020) suggested that applying Zn fertiliser to the soil might significantly decrease the phytate: zinc molar ratio. Previous research Karmakar et al. (2020) has discovered a negative correlation between bioavailable Zn and phytic acid in grain. Yatou et al. (2018) observed that there had been no direct relationship between phytic acid content and Zn content. Various studies revealed close relationships between Zn and other essential nutrient in rice grain (Zhang et al., 2018). The fraction of soluble Zn released from the rice grain after digestion is referred to as bioavailability of Zn. Bioavailability of Zn has been found to be influenced by Zn solubility, digestion phase, and dietary components (Zhang et al., 2020). Wei et al. (2012) also observed that Zn bio-fortification enhanced the quantity of soluble Zn in rice grain, so the total Zn concentration is the most important factor in determining Zn bioavailability. The phytate to Zn molar ratio seems to be a good indicator of Zn bioavailability. The World Health Organization estimates that, When the molar ratio of phytic acid to zinc is more than 15, less than 15% of Zn from food is absorbed (Tsakirpaloglou et al., 2019). This indicated that increasing Zn content while lowering phytic acid concentration in rice grain can improve Zn bioavailability.

Zinc transfer coefficient and plant enrichment factor

The translocation coefficient of Zn in grain and straw were significantly different among the treatment (Figure 2). The maximum TC for grain and straw was reported at T_3, which was 2.27 and 15.2**%** higher over RDF. With rising the level of Zn application, translocation coefficient in grain and straw were significantly reduced. At higher level of Zn application (10.0 mg kg^{-1}), the TC value for grain and straw was 65.7 and 75.6% decreased over RDF. The TC value of Zn for grain was less than straw observed among all the treatment. It was also observed TC value of grain was less than straw. In this case, values higher than 1 indicate that the plant effectively translocate metals from root to the aboveground plant part. The plant enrichment factor of Zn for grain and straw was significantly increased with increased in application rate (Figure 2). It was varied between 0.85-2.50 for grain and for straw was 0.86-3.86. Among all the treatment, the maximum Zn plant enrichment factor was observed at T10 (3.84 for straw and 2.50 in grain)**.** The transfer factor (TC) was indicated the amount of metal accumulated from for soil-to-edible parts of rice. Initially by raising the rate of Zn up to 2.5 mg kg^{-1} after that TC value decreased (Figure 2). This might be due to higher rate of Zn applications get adsorbed in to soil. The plant uptake significant amount of Zn if the TC is greater than 1, TC equal to 1 indicated that the plant uptake optimum amount of Zn and TC less than 1 indicates that the Zn uptake is insignificant level (Olowoyo et al., 2010). It was recorded the plant enrichment factor in straw was higher than grain. Vymazal and Brezinova (2015) reported that Zn Sequestration increased from top to bottom in plant. Plants precultured without a Zn supply had much lower 65Zn translocation than those precultured with an adequate Zn supply, according to a radio-labeled Zn (65Zn) experiment (Gupta et al., 2016; Erenoglu et al., 2011), indicating that plant Zn fertilizer has an impact on Zn translocation to grain. But rate of translocation is higher in control then Zn treated plant.

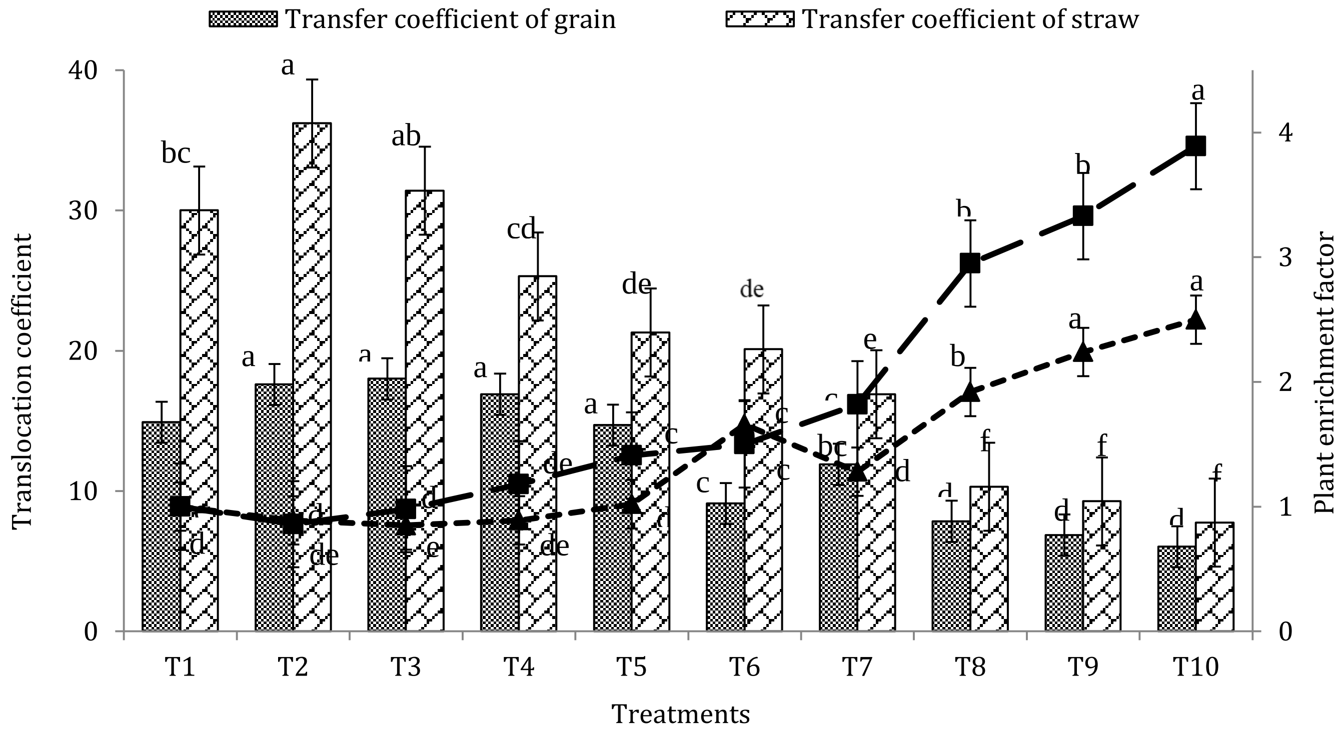

Figure 2. Effect of zinc application on Zn transfer coefficient from soil to grain and straw and plant enrichment factor of Zn in grain and straw of rice (Different letters for each parameter show significant difference at p ≤ 0.05 by Duncan's Multiple Range Test)

Error bars identifies standard error of mean of different treatments

Soil Zn indices

Zn build up index actually indicate the quantity of metal build up in soil matrix. in the present study the buildup factor was found to be increased with increased in Zn application rate. The Zn build up factor varied in between 0.98 – 4.90 among various treatments. Igeo is the quantitative measure used for the assessment of contamination level of Zn through addition of Zn fertilizer. (Figure 3) indicate the Igeo level of all the treatment from control to T_3 fell under the category of unpolluted Class-I for Zn (Igeo < 0), from T_4 –T_6 fell under the class-II (0< Igeo < 1). While remaining treatment fell under moderate pollution categories. Figure 3 illustrated the EF value for Zn in post-harvest Zn treated soil. Across the treatments EF value for Zn was ranged between 0.24-1.82. Zinc was applied to ameliorating the Zn deficiency, biofortification of Zn and higher production. The I-geo values of Zn in paddy soil around were ranged between -0.19 – 1.70 (Class 1 and 2, Antoniadis et al., 2017). At 8.75 and 10 mg kg^{-1} Zn application, I-geo values were around 2, indicating moderate Zn contamination. Lei et al. (2015) also reported that Zn had the highest concentration in paddy soils and its I-geo values ranged between moderates to sever contamination. Soil enrichment factor is helpful in understanding the influence of extreme Zn fertilization. According to the Chen et al. (2017), EF < 1 indicates no enrichment, EF< 3 is minor enrichment. Among these treatments, from control to T7, the EF value fall below <1, while from T_8 to T_9 the EF value was < 3. From this observation it was shown that higher level of Zn treated soil is little toxic to both plant and human. Among all the treatment it came under deficient enrichment. It indicated less hazardous to human being.

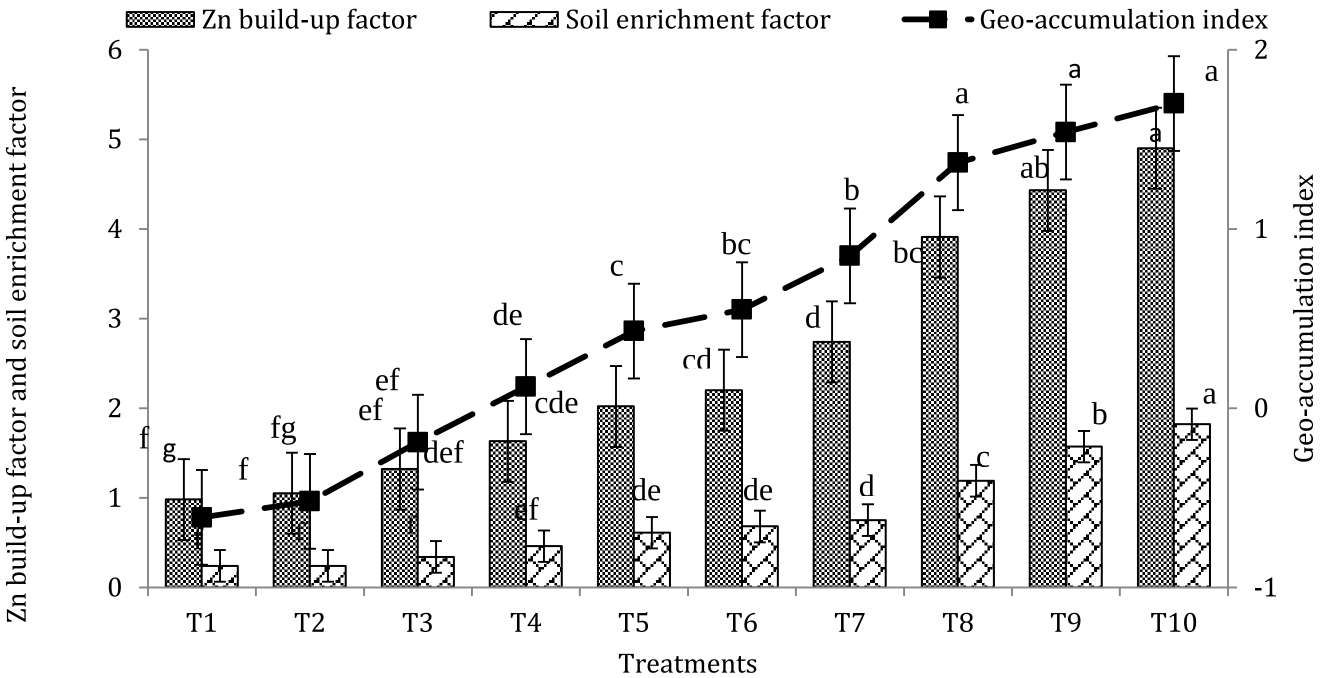

Figure 3. Effect of zinc application on Zn build-up factor, geo accumulation index and soil enrichment factor of zinc in post-harvest soil (Different letters for each parameter show significant difference at p ≤ 0.05 by Duncan's Multiple Range Test) Error bars identifies standard error of mean of different treatments

Dietary intake of Zn

Assuming that an adult man consumes 300 g of cooked rice in their daily diet, the amount of bioavailable Zn (ADI) in cooked rice prepared from Zn treated treatment varied from 0.071 to 0.121 mg kg^{-1} (Figure 4). Among the different treatment, the maximum average daily intake of Zn was recorded at T_7. In T_7, the ADI value of Zn fortified grain was 44% over RDF treated grain. Average daily Zn intake by adult human by consuming Zn fortified grain was presented in the Figure 4. The maximum levels Zn in foods is ≤50.0 mg kg^{-1} dry weight (Ministry of Health, 1991). Daily intakes of Zn in this study through rice were less than the recommended values of Zn for adult, which is 11 mg (Institute of Medicine, Food and Nutrition Board, 2001). It might be due to dietary zinc bioavailability was affected by the phytate content in the grain. Zinc is primary concentrated in the aleurone and embryonic parts of cereals and very small quantity in endosperm. Zinc content in the endosperm round 10 mg Zn kg^{-1}, whereas in embryo and aleurone layer have more than 100 mg Zn kg^{-1} (Cakmak and Kutman, 2018).

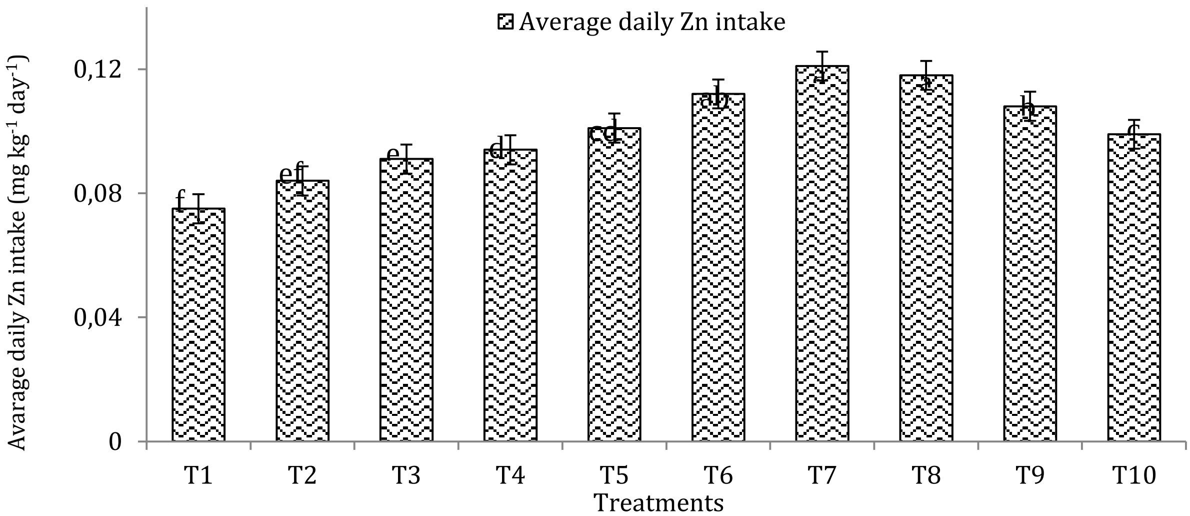

Figure 4. Effect of zinc application on average daily Zn intake by adult human by consuming Zn fortified grain (Different letters for each parameter show significant difference at p ≤ 0.05 by Duncan's Multiple Range Test)
Error bars identifies standard error of mean of different treatments

Correlations

The correlation between among different zinc indices in soil, plant, and human presented in Table 7 at 0.95 and 0.01 confidence levels ($p < 0.05$ and $p < 0.01$) using ANOVA are presented in Table 7. The results show that Pearson correlation with 2-tailed test showed that, at 0.05 level ($p < 0.05$) positive significant relationships existed between average daily intake – grain Zn content (r = 1.00) and 0.01 level ($p < 0.01$) positive significant relationships existed between post-harvest soil Zn content – plant enrichment factor in grain and straw (r = 0.946, 0.989), Zn build-up factor – plant enrichment factor in grain and straw (r = 0.946, 0.989), Geo-accumulation index-grain Zn content (r = 0.756), Geo-accumulation index – plant enrichment factor in grain and straw (r = 0.898, 0.937) and soil enrichment factor – plant enrichment factor in grain and straw (r = 0.951, 0.989). This was suggesting that higher the Zn indices value in soil higher amount of Zn sequestered in plant. At 0.01 level ($p < 0.01$) there is also negative significant correlation between Zn soil indices – transfer coefficient. The negative correlation might be attributed to the higher Zn content in postharvest soil signify less translocation. At level 0.01 level ($p < 0.01$) there is also positive significant correlation between Average daily intake-soil Zn indices (r= 0.551, 0.551, 0.686 and 0.487), Average daily intake-Zn uptake in grain and straw (r = 0.920, 0.681) and Average daily intake was negatively correlated with Transfer coefficient (r= -0.554, -0661, $p < 0.01$).

Table 7. Pearson's correlation coefficients (r) among different zinc indices in soil, plant and human

Parameters	Zn uptake		Phytate content	Total absorbed zinc	Transfer coefficient		Enrichment factor		Average daily intake
	Grain	Straw			Grain	Straw	Grain	Straw	
Soil Zn after harvest	0.37*	0.003	0.14	0.48*	-0.88**	-0.93**	0.95**	0.99**	0.55**
Zn build-up factor	0.37*	0.003	0.14	0.48*	-0.88**	-0.93**	0.95**	0.99**	0.55**
Geo-accumulation index	0.54**	0.190	-0.04	0.63**	-0.89**	-0.97**	0.90**	0.94**	0.69**
Soil enrichment factor	0.31	-0.08	0.24	0.38*	-0.86**	-0.90**	0.95**	0.99**	0.49**
Apparent Zn balance	-0.67**	-0.33	-0.33	-0.70**	0.83**	0.91**	-0.82**	-0.83**	-0.77**
Average daily intake	0.92**	0.68**	0.68**	0.48*	-0.55**	-0.66**	0.45*	0.46*	1

*significance at $p < 0.05$ level and **significance at $p < 0.01$ level

Conclusion

Application of Zn enriched soil on macro- and micro-nutrient uptake in cereal grain, risk assessment of human health and further on the average daily intake. Zn fertilizer application at optimum quantity can achieve the Zn biofortification aim for hybrid rice grain while producing no hazardous impact to soil health. At 6.25 mg kg^{-1} Zn application was a sustainable approach for growth, nutrient uptake, Zn bioavailability and average daily intake of Zn at low economic lost without causing hazardous impact on soil and plant. The current study's findings will assist farmers in optimizing Zn fertilizer management in crop production in the future.

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