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Impact of Zincated nano-clay polymer composites (ZNCPC) and nano ZnO fertilizers on Zn fractionation and its uptake in rice crop of alluvial soils of Indo-Gangetic plains

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ABSTRACT

Zn use efficiency rarely exceeds 1-5%. Grain enrichment of Zn through scientific interventions (microbial and nano-formulation) were studied in a pot experiment using rice (cv. Rajendra mahsuri) as a test crop. Experimental soil was deficient in DTPA extractable Zn (0.48 mg kg^{-1}). $ZnSO_4 \cdot 7H_2O$ with two modes of application (Soil and foliar) was compared with Zn solubilizers (Azospirillum brasilens) and two types of nano formulation (ZnO as spray and Zincated nano-clay polymer composite (ZNCPC) as soil application) at three phonological growth stages of rice (maximum tillering, panicle initiation and harvesting). Zn content in the plant was recorded maximum at panicle initiation stage in ZNCPC. Sequential fractionation study revealed the significant increase in water soluble and exchangeable fractions of Zn in Zn solubilizer (Azospirillum brasilens) and ZNCPC treated soil simultaneously decreasing residual fraction. Apparent zinc recovery was maximum in treatments, ZNCPC (25.56%) followed by nano ZnO sprays (15.25%) and Zn solubilizer (10.76%). Use of ZNCPC, Nano ZnO and Zn solubilizer (Azospirillum brasilens) proved to be promising technology in increasing Zn use efficiency in rice.

Keywords: ZNCPC; Nano-zinc; Zinc solubilizers; Rice rhizosphere; Zn-fractions; Zn-uptake; Zn

INTRODUCTION

Sustainability of crop production in modern scenario due to indiscriminate use of agrochemicals, forfeiture of biodiversity, prompt decline in the ground water level etc. causes multi-nutrient deficiency and affects plant growth (Mandal *et al.,* 2013). Negligence of deficient nutrients esp. micronutrients under intensive cropping system with improved high yielding varieties triggers their deficiency and causes yield loss. Indian soils are deficient in Zn thus, lower Zn inherited in grain content and causes malnutrition to highest order of food web (Cakmak, 2008; Cakmak *et al.,* 2010; Zaman *et al.,* 2018). Application of Zn fertilizers to soils is a general strategy to manage with Zn deficiency and to increase Zn content in grain. However, Zn use efficiency rarely exceeds 1-5 % despite sincere scientific effort globally, which is a major concern for the sustainable crop production. There are group of certain rhizospheric bacteria *viz* Acinetobacter, Bacillus, Gluconacetobacter and Pseudomonas solubilises Zn complex to mineral zinc (ZnO and $ZnCO_3$) make available to growing plants (Gandhi and Murlidharan, 2016; Sachdev *et al.,* 2010). Soil Zn fractions are often distinguished with regard to chemical binding characteristics (Wang *et al.,* 2009). Distribution between the solid fractions also depends on the physico-chemical properties, their bio-availability, mobility and toxicity in plant-soil consortium (Zahedifar, 2017; Baghernejad *et al.,* 2015; Narwal *et al.,* 1999). Plant availability is often associated with water soluble, exchangeable and some extent organic fractions however, rest of the fractions are labile but non-available (Krishnamurti and Naidu, 2008). Water soluble fraction dissociates readily on irrigation while exchangeable are adsorbed by weak electrostatic interaction. Organic bounded are chelated complex which avails at suitable pH (Baeyens *et al.,* 2003; Baghernejad *et al.,* 2015). Rest of the fractions are related with higher ordered chelation and complexations (Baghernejad *et al.,* 2015; Li *et al.,* 2007). Rice rhizosphere precipitate almost Zn content in its mineral forms (Smithsonite, Franklinite etc) thereby reduces Zn use efficiency thus, an innovative technological intervention in rice rhizosphere needs to be explored such as application of Zn at panicle initiation stage results high mobilization from leaves to grain (Khoshgoftarmanesh *et al.,* 2018; Rahman *et al.,* 2012).

Nano-tech is a science as well as art of transforming the matter at atomic or nano-scale ($1nm=10^{-9}$ m), and plays importance role in intervention, formulation and application in nano-fertilizer *viz* - smart delivery of agrochemicals, nano-biosensors, increasing shelf life of fruits and vegetables etc. (Mandal *et al.,* 2015). The development of the intelligent micronutrient fertilizer delivery platforms (IMNDP) may be possible on the basis of elucidating communication signals between plant roots and soil microbes (Monreal *et al.,* 2016). Zincated nano-clay polymer composites (ZNCPC) increases DTPA Zn, Olsen-P, soil enzymatic activities, Zn content and uptake under wheat (Mandal *et al.,* 2015; Mandal *et al.,* 2018-a; Mandal *et al.,* 2019). Comparative evaluation of nano Zn carriers along with Zn solubilizers, effect on changes in various Zn fractions under various phenological growth stages of rice need to be undertaken for understanding dynamics of nano Zn sources in soil-plant consortium. Keeping this view, an experiment has been embarked to investigate the efficacy of different Zn sources, changes in sequential Zn fractions its relationship with Zn content in soil as well as uptake in increasing Zn use efficiency of transplanted rice crop.

MATERIALS AND METHODS

Soil sampling, collection and chemical analysis

The initial soil samples were collected from experimental site with help of a spade from randomly selected places from the University research farm, BAU, Sabour. Soil sample was air dried, sieved through a 2mm sieve and stored in good quality polythene containers. The polythene contained soil samples were initially analyzed for physico-chemical properties including pH, EC, OC, Olsen-P, NH_4OAC-K and DTPA extractable Zn content (Table 1).

Table 1: Initial Physical and Chemical analysis of Experimental site

Parameter	Value	Method employed
pH (1:2.5)	7.8	Jackson, 1973
EC (dSm^{-1})	0.20	Jackson, 1973
Soil moisture (%)	45	He, *et al.,* 2021
Alkaline $KMnO_4$ N (kg ha^{-1})	125.44	Sibbiah and. Asija., 1956
Olsen- P (kg ha^{-1})	12.55	Olsen *et al.,* 1954
1 N NH_4OAC K (kg ha^{-1})	240.92	Hanway and Hiedal, 1952
DTPA Zn (mg kg^{-1})	0.48	Lindsay and Norvell, 1978
Organic carbon (%)	0.47	Walkley and Black, 1934
CEC cmol (P+) kg^{-1} soil	18.57	Adamu *et al.,* 1989
Mechanical analysis (%)	-	Bouyoucos, 1962
Sand	50.8	-
Silt	40	-
Clay	9.2	-
Texture	Sandy loam	
Nomenclature according to 7th approximation	*Typic Haplustepts*	

Cultivation of the crop

The experiment was conducted in the net house with viable and healthy seedlings of rice (Oryza sativa, cv- Rajendra mahsuri) in pots having 10 kg soil. The recommended NPK were used as Urea (24.26 ml pot $^{-1}$), KH_2PO_4 (21.90 ml pot $^{-1}$) and KCl (7.10 ml pot $^{-1}$).

The treatments were laid in factorial completely randomized design with six treatments viz – 0 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1} (T_1), 25 kg $ZnSO_4 \cdot 7H_2O$ ha^{-1}(T_2), 1% $ZnSO_4 \cdot 7H_2O$ with lime spray (T_3), 25 kg $ZnSO_4.7H_2O$ ha^{-1} with Azospirilum brasilense (14×10^3 g^{-1} dry soil) (T_4), 40 ppm nano ZnO spray (T_5) and ZNCPC (T_6). $ZnSO_4 \cdot 7H_2O$ with lime spray and Nano Zinc spray were imposed at maximum tillering stage after maintaining the pot moisture at field capacity (18% moisture content).

Plant sampling and analysis

Plant samples were taken at three different growth stages maximum tillering stage (S1), panicle initiation stage (S2) and harvesting stage (S3). For analysis, plants were harvested by cutting, washed thoroughly, kept in shade for air drying and oven dried at 65±1°C till constant weight (biomass yield) and ground thoroughly by a Wiley mill. Samples (1.0 g) were pre-digested in conical flasks (100 mL capacity) with 5 mL of concentrated HNO_3 and finally digested in a di-acid mixture (8 mL) containing HNO_3 and $HClO_4$ acid (9:4) on an electric hot plate following standard procedure (Piper *et al.,* 1967). The digested material was cooled, diluted with distilled water and filtered through Whatman No. 1 filter paper. Volume was made up to 100 mL and stored in a polypropylene container for further analysis.

ZnO nanoparticle and Zincated nanoclay polymer composites (ZNCPC) synthesis and characterization

ZnO nano-particles were synthesized as per Aneesh *et al.,* 2007. 0.1M Zn $(CH_3COO)_2 .2H_2O$ stock solution was prepared in 50 mL methanol under stirring. The zincated methanol working solution was prepared at 10 pH by adding 25mL of 0.2 M NaOH. Desired solution were transferred into teflon lined sealed stainless steel autoclaves and maintained at 150℃ for 6 hour under autogenous pressure and allowed to cool naturally to room temperature. ZNCPC controlled released nano-Zn fertilizers composed of nanoclay as diffusion barrier, acrylic acid and acrylamide monomers, N, N, Methylene Bisacrylamide crosslinker and ammonium persulfate initiator. This is an exfoliated type nano-composite because of disappearance of typical bentonitic peak (11.49 Å) (Fig 1). Exfoliation results dispersion of clay into multiple small pellets. Zn-citrate was used as carrier of Zn within ZNCPC containing 6.59% Zn. Formation of ZNCPC was outlined by Mandal *et al.,* 2018-b.

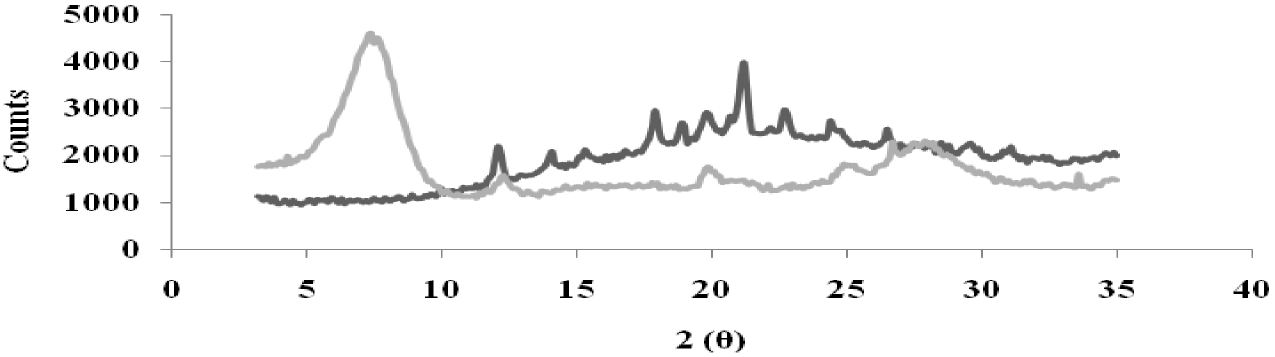


Figure 1: X-ray diffractogram of Nano Clay Polymer Composites (NCPC) based Zn

ZnO nanoparticle and ZNCPC were characterized through TEM and XRD. TEM images of ZnO and nano-clay nano-particle were taken through a TEM made by JEOL, Japan (JEN 1011, 100 KV). Specific instrumental set-up was 80 kV HV with magnification 80000 X. However, in XRD the powder diffractions of ZnO and nano-clay were taken and scanned separately in a Philips PW 1710 X-ray diffractometer, using automated powder diffraction software (PAN analytical, Spectris Technologies) with the setting of the instruments as hereunder : Radiation type: Cu Kα; Tube current : 20 mA ; Generator voltage: 40 kV; Start angle (°2θ): 3.00; End angle (°2θ): 40.00; Scan step size: 0.1; Scan type: Continuous; Scan speed (2θ Sec^{-1}): 0.025 (Fig 1).

Zinc fractionation study

To study the distribution of Zn among various binding forms, the sequential fraction procedures with 1.5g sample mass were used in each step (Iwasaki and Yoshikawa, 1990; Miller *et al.,* 1986).

The Zn fractionation schemes were as follows:

- Water soluble Zinc: 25 mL H_2O were shaken for 16 h.
- Exchangeable Zinc: 25 mL 0.5 M calcium nitrate $[Ca(NO_3)_2]$-solution was shaken for 16h.
- Specifically absorbed [lead (Pb)-displaceable fraction] Zinc: 25 mL of a solution of 0.05 M lead nitrate $[Pb\ (NO_3)_2]$ and 0.5 M ammonium acetate at pH 6.0 were shaken for 2 h.
- Acid-soluble fraction Zinc: 25 mL of 2.5% acetic acid were shaken for 2 h.
- Manganese-oxide-bound fraction: 50 mL of 0.1 M hydroxylamine hydrochloride solution at pH 2.0 were shaken for 30 min.
- Organic-matter-bound fraction: 50 mL of 0.1 M potassium pyrophosphate solution at pH 10.0 were shaken for 2 h.

- Amorphous iron-oxide-occluded fraction: 50 mL of 0.1 M oxalic acid solution and 0.175 M ammonium oxalate [$(NH_4)_2C_2O_4$] solution at pH 3.25 were kept for 4 h in the dark.
- Crystalline iron-oxide-occluded fraction: 50 mL of 0.1 M oxalic acid solution, 0.175 M $(NH_4)_2C_2O_4$ solution (ammonium oxalate), and 0.1 M ascorbic acid was kept for 30 min in a boiling water bath.
- Residual fraction: The final soil residue was then digested in an $HF\text{-}HClO_4$ acid mixture.

Mobility Factor (MF)

Some of the aforementioned six fractions are very mobile in nature and can be estimated according to absolute and relative content of relative weakly bounded fractions. MF is the indication of potential movement of metal of contaminating medium (Kabala and Singh, 2001) as follows:

$$MF = \frac{F_1 + F_2 + F_3 + F_5}{F_1 + F_2 + F_3 + F_4 + F_5 + F_6 + F_7 + F_8 + F_9} \times 100$$

Where F_1 – Water soluble fraction; F_2 – Exchangeable fraction; F_3 – Acid soluble fraction; F_4 – Magnese oxide fraction; F_5 – Organically bounded fraction; F_6 – Specifically absorbed; F_7 – Amorphous iron-oxide-occluded fraction; F_8 – Crystalline iron-oxide-occluded fraction; F_9 – Residual fraction.

The values of MF of heavy metals suggest the affinity rate of Zn in food chain. If $MF < 1$considered as no risk, $1 < MF < 10$ – low risk; $10 < MF < 30$ – medium risk; $30 < MF < 50$ – high risk; $50 < MF < 75$ – severe risk (C. Kabala and B.R. Singh, 2001).

Total Zn and Apparent Zn recovery

Mathematically, total zinc (mg kg^{-1}) and apparent Zn recovery (%) were calculated as follows:

$$\text{Zn uptake } (\text{mg kg}^{-1}) = (\text{Zn content in grain} + \text{Zn content in straw}) \text{ x biological yield}$$

$$\text{Apparent Zn recovery } (\%) = \frac{\text{Zn uptake in applied pot} - \text{Zn uptake in control}}{\text{Amount of Zn applied}} \text{ x } 100$$

Statistical analysis

The significant differences among the treatment means were calculated at 5% and 1% at probability levels $p \leq 0.05$ and <0.01 respectively using Excel (Microsoft, USA) and SPSS (SPSS, USA) software packages (Gomez and Gomez, 1984).

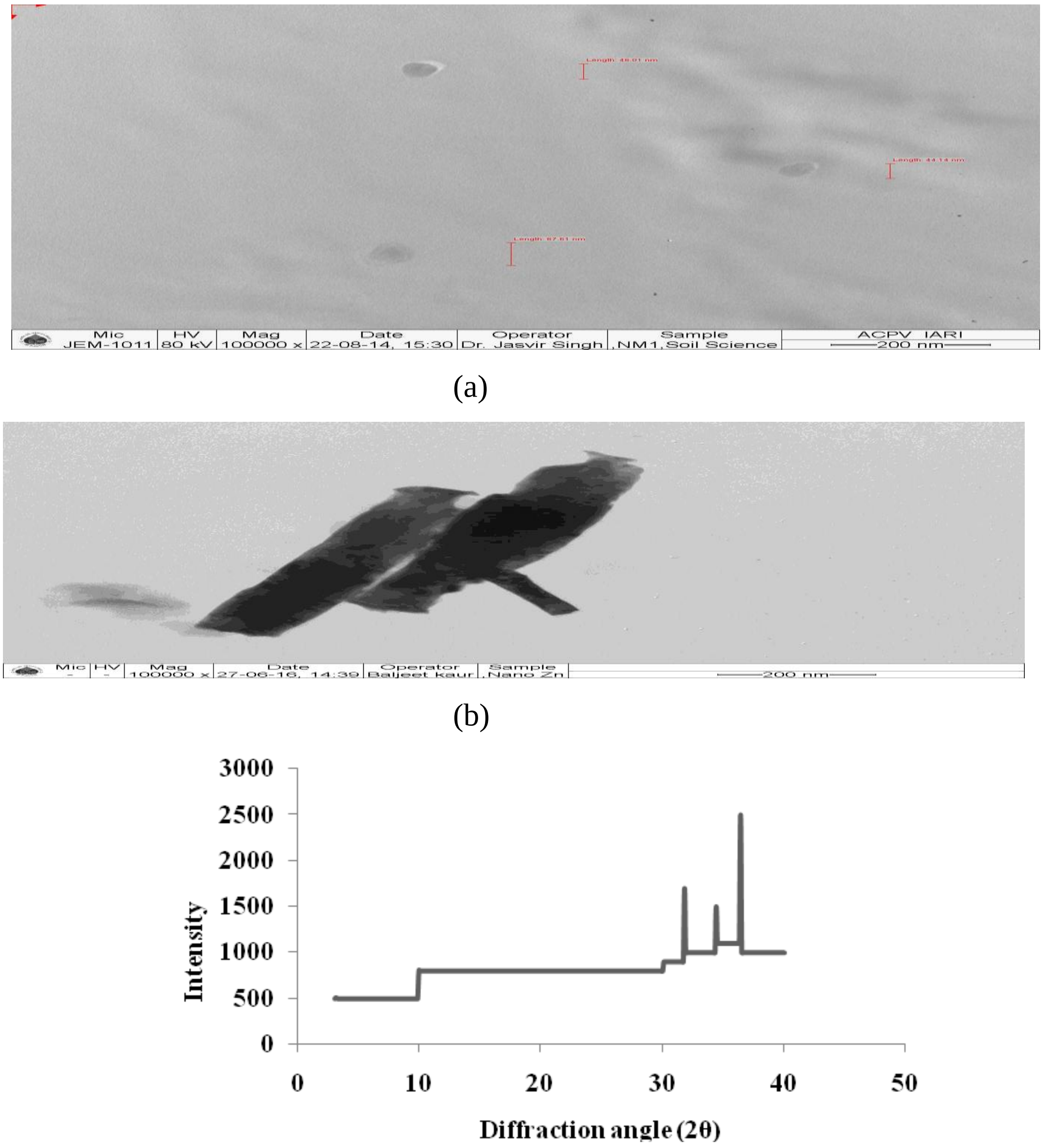
Regression and correlation analysis was also carried out to find out relationship among various parameters and presented in graphical form.

RESULTS AND DISCUSSION

Effect of different treatments on DTPA extractable Zn content in soil and plants

Nanoclay polymer composites (NCPC) acts as rhizosphere controlled release fertilizer formulations (Mandal *et al.,* 2018-b). Transmission electron microscopy image confirmed that clay was in nano-size (Fig. 1). X-Ray diffractogram (XRD) proved that the clay used were montmorillonite (12.6 Å first order peak) (Fig. 2). Release of Zn in soil solution from ZNCPC occurred through microbial mediated enzymatic breakdown of multiple linkages present within ZNCPC (Mandal *et al.,* 2018-b). Hence, relatively higher content of Zn in T_6 across different crop growth stages might be explained by slow release of Zn from ZNCPC thereby masking its interaction in ionic form with negatively charged clay particles which otherwise make complex with solution Zn resulting lower availability of this important micronutrients (Bais *et al.,* 2001). ZNCPC increased Zn content in soil by two fold action (i) slow release of Zn into solution (ii) release of citrate ion concomitantly in solution solubilising native or applied Zn present therein. Other researcher also observed a 9.7-fold increase in Zn solubility by applying root exudates to soil. Zn content in soil under conventional fertilizer treatment ($ZnSO_4 \cdot 7\ H_2O$, soil application) was lower owing to higher solubility of sulphate salts and interaction of Zn^{2+} with soil components particularly clay and organic matter (Alloway, 2009).

The maximum DTPA Zn in soil and plant at maximum tillering stage was found to be highest in T_4 (2.65 mg kg^{-1} in soil, 29 mg kg^{-1} in plant) however, at panicle initiation stage it results highest at T_6 (3.60 mg kg^{-1} in soil, 33.33 mg kg^{-1} in plant) and at the harvest stage T_6 (3.50 mg kg^{-1} in soil, 37.67 mg kg^{-1} in plant) (Table 2). The variation is due to solubilisation of native or applied Zn by low molecular weight organic acid (such as citrate, malate, oxalate etc) which increases phyto-availability in rhizosphere and involved in internal metal



(c)

Figure 2 (a) – TEM of nano-Zn particles, (b) – morphology, (c) – XRD of nano-Zn particles

fractional study revealed that at panicle initiation stage in T_4, there was reduction in residual Zn (19%) whereas water soluble and exchangeable Zn increased by 2% each. The increase in exchangeable zinc might due to increase in the rate of mineralization of organic complexes and bound zinc from recalcitrant sources such as carbonates of zinc into exchangeable zinc under Bacillus aryabhattai inoculation (Ramesh *et al.,* 2014).

High-molecular-weight organic acids reduces the bioavailability and toxicity of heavy metals, while low-molecular-weight organic acids and amino acids can increase the bioavailability and plant accumulation of heavy metals through

Amorphous iron-oxide-occluded, specifically absorbed [lead (Pb)-displaceable fraction] and Residual Zn fraction

The mean values of amorphous iron-oxide-occluded, specifically absorbed [lead (Pb)-displaceable fraction] and residual Zn fraction varies as 1.64, 3.69 and 2.33% respectively on the application of different combination of Zn fertilizers. In amorphous iron-oxide-occluded and residual Zn fraction the control treated pot results atpar with some treatment. Amorphous iron-oxide bounded Zn fraction results highest concentration in $T_2>T_4>T_1>T_6>T_5$ and least in T_3. However, specifically absorbed [lead (Pb)-

Table 2: Effect of treatments on DTPA extractable Zn in soil and plants (mg kg^{-1}) at different growth stages of rice plants

Treatment	DTPA extractable Zn in soil (mg kg^{-1})				DTPA extractable Zn in plant (mg kg^{-1})			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
T_1	0.50	0.73	0.64	0.62	16.67	19.33	22.00	19.33
T_2	0.76	1.05	0.98	0.93	22.33	21.67	24.33	22.78
T_3	0.58	1.59	1.54	1.24	20.67	28.67	32.67	27.33
T_4	2.65	3.52	3.46	3.19	29.00	25.33	35.00	29.78
T_5	0.64	1.39	1.43	1.15	21.00	33.00	36.67	30.22
T_6	2.63	3.60	3.50	3.24	27.67	33.33	37.67	32.89
Mean	1.02	1.98	1.92	1.73	22.89	26.89	31.39	27.06
Particulars		SE $_{m(\pm)}$	CD 5%	CD 1%		SE$_{m(\pm)}$	CD 5%	CD 1%
Stage (S)		0.05	0.13	0.18		0.71	2.04	2.74
Treatment (T)		0.06	0.19	0.25		1.01	2.89	3.87

S_1 = Maximum tillering stage, S_2 = Panicle initiation stage and S_3 = Harvesting stage

displaceable fraction] varies as $T_2>T_3>T_5>T_4=T_6>T_1$ and residual varies as $T_2>T_1>T_3>T_5>T_4>T_6$ (Table 3).

Dynamics of Zn fractions at different stages of sampling

Acid soluble specifically absorbed, amorphous and manganese oxide Zn fraction contributes highest at harvesting stage whereas crystalline and organic fraction comprises maximum Zn fraction at initial stage. The water soluble and exchangeable Zn fraction optimizes at panicle initiation stage and residual fractions results same in both initial and panicle initiation stages (Fig 3 b). In all the three stages residual fraction contributes to maximum while water soluble fraction contributes to minimum (Fig 3 a).

Table 3: Effect of different treatment on soil sequential fractionation of Zn at harvesting stage

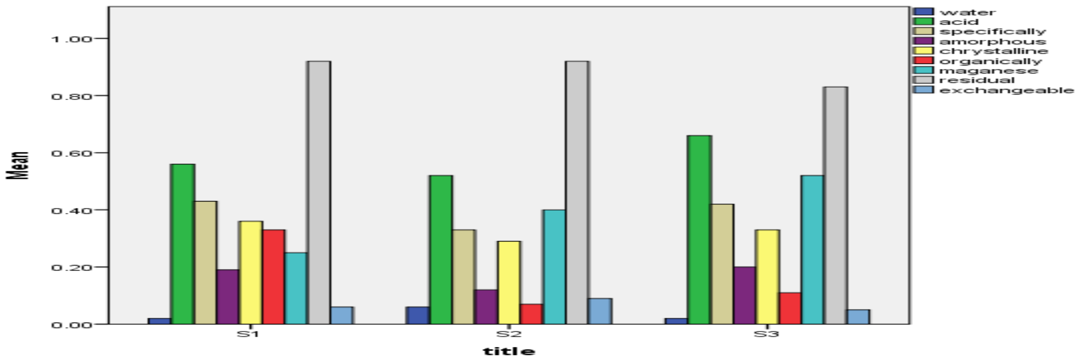
Treatment	Sequential fractionation of Zn										
	Wtr –so (mgkg^{-1})	Ex (mg kg^{-1})	Acd –sol (mgkg^{-1})	MnO_2 Bound (mgkg^{-1})	Om – Bound (mgkg^{-1})	Al-Fe-Bound (mg kg^{-1})	Crystalline (mg kg^{-1})	Spec-absorbed (mg kg^{-1})	Residual (mg kg^{-1})	Total (mgkg^{-1})	Mobility Fraction
T_1	0.010	0.015	0.350	0.320	0.350	0.330	0.195	0.230	0.670	2.470	15.18
T_2	0.019	0.029	0.400	0.480	0.560	0.710	0.230	0.560	0.820	3.808	15.97
T_3	0.025	0.040	0.620	0.420	0.250	0.230	0.350	0.350	0.560	2.845	11.07
T_4	0.030	0.053	0.750	0.650	0.650	0.620	0.580	0.310	0.410	3.690	18.09
T_5	0.022	0.023	0.450	0.320	0.320	0.310	0.450	0.330	0.530	2.755	13.25
T_6	0.040	0.060	0.950	0.350	0.590	0.320	0.720	0.310	0.410	3.750	18.4
CD (P=0.05)	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	-
CV (%)	3.83	4.63	1.23	2.73	2.64	1.64	5.67	3.69	2.33	1.18	
SEm (+-)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	-

Mobility factor

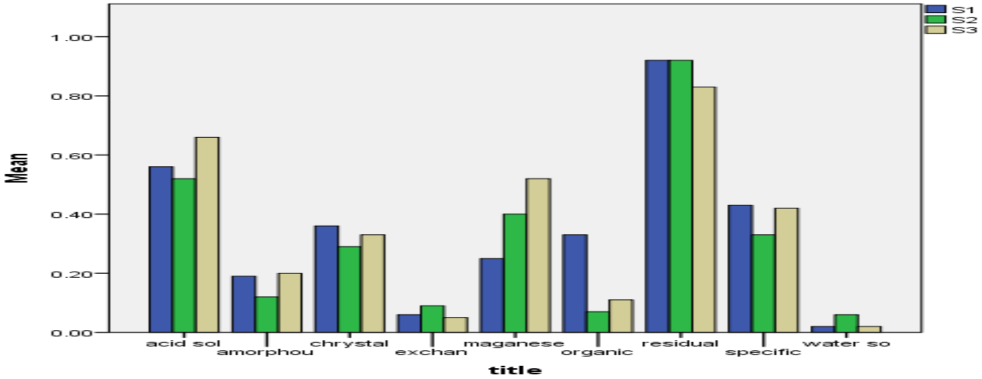
Application of Zn at various treated paddy pots ranges MF between 11.07 (T_3) to 18.40(T_6) with an average 15.33 shows medium risk of Zn mobilization (Kabala and Singh, 2001). MF follows sequence $T_6>T_4>T_2>T_1>T_5>T_3$. In Zn mobilization T_1 results atpar with T_3 and T_5. Increase in mobility nature from treated pots results the Zn availability which is being up taken by plants and increase its biomass yield. Higher Zn content in water soluble and exchangeable Zn fraction in T_6 might due to controlled release of Zn from NCPC and citrate solubilisation of native Zn present in soil (Simine *et al.,* 1998). Reduction of residual Zn fraction in T_4 (RDF+ Azospirillum) might be due to low molecular weight organic avid secretions which solubilised native or applied Zn (Impa *et al.,* 2012).

Effect of treatments on biomass yield, total Zn (mg kg^{-1}) uptake and content in grain of rice plants at different stages

The significant increase in biomass yield in all the treatment as compared to control was



(a) – stage wise fractionization of Zn



(b) – comparative study of different Zn fractions at three stages of paddy crop

Figure 3: Dynamics of Zn fractions

recorded on application of Zn fertilizers. Biomass yield in plant at maximum tillering stage was found to maximum in T_6 (3.95 g kg^{-1}) however at panicle initiation stage and harvesting was out yielded by T_6 6.81 g kg^{-1} and 9.14 g kg^{-1} respectively (Table 4). Total zinc content in the grain and uptake both observed highest in T_6 as 40.00 mg kg^{-1} and 303.42 mg kg^{-1} followed by $T_5 > T_4 > T_3 > T_2 > T_1$ and $T_4 > T_5 > T_3 > T_2 > T_1$ respectively. However, there was significant increase in total zinc content in grain in all the treatment as compared to control but Zn uptake varied non-significantly (Table 4).

Table 4: Effect of treatments on biomass yield (g kg^1soil) at different growth stages of rice plants

Treatment	S_1	S_2	S_3	Mean
T_1	2.34	4.99	6.64	4.32
T_2	2.93	5.75	6.24	4.97
T_3	2.89	5.61	6.57	5.02
T_4	3.46	6.46	7.57	6.30
T_5	2.83	5.53	7.67	5.27
T_6	3.95	6.81	7.84	6.63
Mean	3.06	5.86	7.33	5.42
Particulars		SE $_{m(\pm)}$	CD at 5%	CD at 1%
Stage (S)		0.17	0.48	0.64
Treatment (T)		0.24	0.68	0.91

S_1 = Maximum tillering stage, S_2 = Panicle initiation stage and S_3 = Harvesting stage

ZNCPC maintained relatively higher amount of Zn content in soil solution across the crop growth stages owing two its two fold actions (Controlled release and citrate solubilisation) resulting significantly higher Zn economy (Bais *et al.,* 2001). Nano ZnO foliar spray recorded higher Zn content at panicle initiation and harvesting stage owing to its direct absorption (nano size) though foliage and translocation to grain. Zn solubilizers (Azospirillum) proved to be effective in maintaining relatively higher Zn content in rhizosphere soil owing to the secretion of low molecular weight organic acids and maintaining higher microbial activity (Increase in dehydrogenase activity) which facilitated solubilisation of native or applied Zn (Impa *et al.,* 2012). Inoculated ZSB (Zinc solubilizing bacteria) isolates solublize the unavailable source of zinc at the maximum when there was high source of insoluble zinc in soil (Gandhi and Murlidharan, 2016).

Effect of various Nano zinc sources with zinc mobilizers on Apparent Zinc Recovery (%)

Apparent zinc recovery was found to be maximum in T_6 (ZNCPC) (25.56%) followed by

Table 5: Effect treatments on total Zn (mg kg^{-1}) content in grain, Zn uptake (mg kg^{-1}) and Apparent Zinc Recovery (%)

Treatment	Zn content (mg kg^{-1})	Zinc uptake (mg kg^{1})	Apparent Zinc Recovery (%)
T_1	9.67	134.50	-
T_2	17.33	154.69	2.23
T_3	21.00	186.29	3.14
T_4	25.00	275.30	10.76
T_5	34.00	214.36	15.25
T_6	40.00	303.42	25.56
Particulars			
SE $_{m(\pm)}$	0.90	-	-
CD at 5%	2.78		
CD at 1%	3.90	-	-

T_5 (15.25%), T_4 (10.76%), T_3 (3.14%) and T_2 (2.23%) (Table 5). Similar trends were observed for Zn content in grain and uptake by plants. Foliar application of conventional Zn sources (T_3) results higher Zn content in biomass and grain as compared to soil applications as it was directly absorbed through foliage.

Table 6: Pearson's correlation matrix

	A	C	D	E	F	G	H	I	J	K	L	M	N	O	P
A	1														
C	-.667	1													
D	-.752	.816*	1												
E	-.454	.803	.901*	1											
F	-.528	.871*	.477	.426	1										
G	-.261	.485	.774	.772	.148	1									
H	-.621	.708	.966**	.893*	.331	.899*	1								
I	-.510	.789	.912*	.889*	.497	.916*	.954**	1							
J	-.592	.637	.951**	.870*	.242	.909*	.993**	.925**	1						
K	-.544	.651	.906*	.809	.331	.950**	.973**	.967**	.967**	1					
L	.339	.251	-.224	-.021	.490	-.045	-.187	.091	-.280	-.101	1				
M	-.566	.544	.763	.583	.324	.844*	.853*	.867*	.832*	.925**	.021	1			
N	-.895*	.770	.773	.604	.573	.348	.687	.626	.625	.594	-.040	.635	1		
O	-.593	.811	.872*	.935**	.454	.533	.785	.723	.761	.639	-.175	.392	.687	1	
P	-.348	.839*	.657	.853*	.608	.363	.562	.612	.504	.429	.229	.198	.556	.896*	1

***Correlation is significant at the $p \leq 0.01$, *Correlation is significant at the $p \leq 0.05$ level, Where A=pH, C=CEC, D=DTPA extractable Zn, E= water soluble Zn, F= exchangeable Zn, G= acid soluble Zn, H=Specifically adsorbed Zn, I= manganese oxide occluded Zn, J=organically bound Zn, K=aluminium iron oxide occluded Zn, L=crystalline iron oxide occluded Zn, M=residual or mineral form Zn, N= zinc content in grain (mg kg^{-1}), O= zinc uptake (mg kg^{-1}), P= biomass yield*

Relationship between DTPA extractable zinc in soil and total zinc content in plant in all growth stage

The study reveals that DTPA extractable Zn is significantly correlated with cation exchange capacity (r^2 = 0.816), water soluble (r^2 = 0.901), specifically absorbed (r^2 = 0.966), MnO-bounded fractions (r^2 = 0.912), organically bounded (r^2 = 0.951), Fe/Al oxide bounded (r^2 = 0.906) and Zn uptake (r^2 = 0.872) (Table 6). This clearly indicates that these fractions are responsible for the availability of available Zn to which plant uptakes. These fractions on the lowering of Zn concentration is being supplied mainly by acid soluble fractions, mineral fractions and plant biomass fraction as per correlation Table 6.

CONCLUSIONS

DTPA Zn availability in soil, active

microbial activity, high organic carbon content and moisture availability results highest apparent Zn recovery for ZNCPC. Synergistic interactions among several parameters resulted significantly higher apparent Zn recovery for ZNCPC based Zn application. ZNCPC proved to be promising Zn fertilizer formulation under rice rhizosphere for increasing Zn use efficiency. Efficacy of ZNCPC needs to be evaluated under long-term experiments via-a-vis $ZnSO_4$. 7 H_2O for the environmental sustainability and economically benefit: cost ratio and large scale farmers' adaptability.

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