Effect of various levels, sources of Zn and Zn solubilizing bacteria on Zinc transformation in calcareous soil

ABSTRACT

Intensive cultivation with high yielding varieties and imbalanced fertilization using high analysis fertilizers resulted in increasing micronutrient deficiencies in soil. Zinc is one among the micronutrients essential for the growth and development of crops. Application of inorganic zinc fertilizers to mitigate Zn deficiency is relatively ineffective since it gets converted into unavailable forms. To study the Zn transformation in calcareous soil as influenced by various levels, sources of Zn and Zn solubilizing bacteria, an incubation experiment was carried out. Distribution of Zn fractions in the soil at harvest stage followed the order WS-Ex < organically bound < carbonate bound < Fe-Mn oxide bound < residual Zn. Plant available forms of Zn viz., water soluble and exchangeable and organically bound Zn were significantly affected by the sources, levels of Zn and ZSB application. Application of $ZnSO_4$ at 7.5 kg Zn ha^{-1} along with ZSB registered high WS-Ex. And Org. Zn. Lower carbonate bound Zn fraction was observed with ZSB application when compared to the treatments without ZSB application. Fe-Mn oxide bound fraction of Zn was also significantly influenced by the sources, levels of Zn and ZSB application. Sources of Zn and ZSB application did not have significant influence on residual and total Zn. With increasing levels of Zn significant increase in all the Zn fractions and total Zn were observed and the highest value being recorded with 7.5 kg Zn ha^{-1}.

Key words: Calcareous soil, Zn level and Sources, Zinc Solubilizing Bacteria, Zn Transformation.

INTRODUCTION

Zinc (Zn) is a trace element found in varying concentrations in all soils, plants and animals and it is essential for the normal healthy growth of higher plants, animals and humans. Zn is important for photosynthesis and respiration and deficiency of zinc decreases the photosynthetic rate, chlorophyll content, activity of carbonic anhydrase and biosynthesis of protein (Cakmak, 2008; Zhang *et al.*, 2016).

Around 51.2 per cent of Indian soils are Zn deficient (Shukla, 2021) it is expected to increase by 63 per cent by 2025 as most of the marginal soils are showing response to added zinc (Singh et al., 2009).. Even though deficiency of Zn is most common in high pH calcareous soils (Katyal and Vlek, 1985), it has also been reported in heavily weathered acid soils (Alloway, 2004; Behera *et al.*, 2011).Cereal crops suffers owing towidespread Zn deficiency and results in low food production and Zn malnutrition. Cakmak (2008) reported that Zn was considered as the fifth most important risk factor responsible for illness and death in the developing world.

Low solubility of Zn rather than low total content of Zn is usually considered as the major reason for the deficiency of Zn in most agricultural soils. Cereal crops are majorly affected by Zn deficiency. It is estimated that nearly half of the cereal growing soils are affected by Zn deficiency. Among the plants, maize (*Zea mays L.*) is highly susceptible to Zn deficiency, particularly in calcareous soils, where high pH and high $CaCO_3$ content affect the Zn availability. Zn deficiency hinders maize growth resulting in decreased grain yield and quality (Behera *et al.*, 2015). Maize is considered as a good indicator crop of low soil Zn levels and in many countries, it receives the highest proportion of Zn fertilizer application.

Globally Calcisols spread over about 800 mha. Surface coverage of lime affected or naturally found calcareous soils in the earth is estimated to be almost greater than 30 per cent (Hu *et al*., 2018). The extent of calcareous soil in India is about 228.8 m ha (69.4 % of total area) and in Tamil Nadu it accounts for 3.70 m ha (28.4 % of total area)(www.gis.nic.in). Around 90-99 per cent of applied Zn fertilizer is adsorbed on soil colloids and precipitated in calcareous soil (Saravanan *et al*., 2004).

Since zinc solubilizing bacteria (ZSB) have a great potential to solubilize the insoluble Zn sources like $ZnCO_3$, ZnO, $Zn_3(PO_4)_2$, its application along with other Zn sources could be effective in making the Zn available to plants, especially when applied to Zn deficient calcareous soil.The solubility of Zn is highly dependent on soil pH and moisture and hence arid and semi arid areas of Indian agro ecosystems are often Zn deficient.

Xiang *et al.* (1995) observed the transformations of the added Zn from the exchangeable, the weakly bound to organic matter and the amorphous iron oxide fractions into the strongly bound to organic matter and the crystalline iron oxide fractions in calcareous soils. Prasad *et al.* (1995)through a greenhouse study with maize as the test crop in calcareous soil reportedthat different fractions of Zn increased with increase in the level of Zn and was in the order carbonate bound Zn > organically bound Zn >complexed Zn> water soluble + exchangeable Zn >HCl soluble Zn.

Transformation of Zn in three different soils having low, medium and high Zn, showed that among the fractions, the higher release of water soluble plus exchangeable Zn (WSEX-Zn), organically bound Zn (OM- Zn) and crystalline bound Zn (CFeOX-Zn) were observed at 15 days after incubation (DAI) and gradually declined at the end of the experiment upto 30 DAI whereas other formssuch as manganese oxide bound zinc (MNOX- Zn), amorphous sesquioxide bound Zn (AFeOX- Zn), residual Zn (RES- Zn) and total- Zn showed a prolonged release upto 30 DAI (Preetha and Stalin, 2014).

Ramesh *et al.* (2014) reported that inoculation of bacteria strains MDSR7, MDSR11 and MDSR14 increased exchangeable zinc whereas depleted organically bound and carbonate bound Zn compared to the uninoculated control. Suganya and Saravanan (2017) in a field experiment conducted in black soils with maize opined that ZSB inoculation increased the Zn content at all the stages of observation.

MATERIALS AND METHODS

An incubation experiment was conducted to investigate the Zn transformation in calcareous soil as influenced by various levels, sources of Zn and Zn solubilizing bacteria. The experiment was conducted in black calcareous soil collected from Eastern block, TNAU Coimbatore with two sources ($ZnSO_4$ and $Zn_3(PO_4)_2$), three levels of Zn(0, 5, 7.5 kg ha^{-1}), with and without Zn solubilizing bacteria. Hundred grams of processed, unsterilized soils were weighed separately into polythene containers and mixed with FYM at the rate of 12.5 t ha^{-1} and calculated quantities of $ZnSO_4$ and $Zn_3(PO_4)_2$ as per treatment schedule. Soil samples were adjusted to field capacity with sterile distilled water.The ZSB strains prepared as inoculum was thoroughly mixed with soil with a final concentration of about 10^8 cfu per g. The experiment was conducted for 90 days. The moisture content was maintained during the course of experiment by correcting the water loss periodically using sterile distilled water.

There are twelve treatments which include two sources of zinc (Zinc sulphate and Zinc phosphate) as Factor 1; three levels of Zinc (0, 5, and 7.5 kg Zn ha^{-1}) as Factor 2 and treatment with zinc solubilizing bacteria (with ZSB and without ZSB) as Factor 3. The treatments were accommodated in Factorial Completely Randomized Design in three replications.

Destructive soil sampling was carried out on 15, 30, 60 and 90 days after incubation and the soil samples were shade dried and analyzed for Zn fractions Table 1.

Table 1. Sequential extraction procedure used for the fractionation of soil Zn

Zn forms	Extractants	Soil /solution ratio(g:mL)	Shaking time (Hours)
Exchangeable and soluble	1M $Mg(NO_3)_2$	1:4	2
Carbonate bound	1M NaOAc (pH=5,CH_3COOH)	1:4	5
Organically bound	0.7 M NaOCl (pH=8.5)	1:2	0.50 (in boiling water bath)
Fe-Mn oxide bound	0.175M $(NH_4)_2C_2O_4$+ 0.1M $H_2C_2O_4$	1:40	4
Total	18.75 ml HCl + 6.25 ml HNO_3	1:25	Digestion with Aqua regia

(Elliott *et al.*, 1990)

The data obtained from the investigations were subjected to the analysis of variance to find out the significance as suggested by Panse and Sukhatme(1978). Wherever the treatment differences were found significant critical differences (CD) were worked out at 5% level of significance with mean separation by least significant difference and denoted by symbol *and ** for 5% and 1%. Non-significant comparisons were indicated as NS. Simple correlation was worked out between different parameters to know the relationship exists among them.

RESULTS

Zinc fractions in calcareous soil

The different fractions of zinc *viz.*, water soluble plus exchangeable, carbonate bound, organically complexed, iron-manganese oxide bound, residual and total zinc were determined in soil. Distribution of Zn fractions in the initial soil followed the order WS-Ex (0.37 mg kg^{-1}) < organically bound (1.45 mg kg^{-1}) < carbonate bound (5.21 mg kg^{-1})<Fe-Mn oxide bound (6.23 mg kg^{-1}) < residual Zn (81.1 mg kg^{-1}).

Water soluble and exchangeable Zn (WS-Ex. Zn)

The results indicated a significant difference in the water soluble and exchangeable Zn with the application of different sources of Zn at 15, 30 and 90 DAI (Table 2). Among the different sources of Zn, $ZnSO_4$ was found to give the highest value of 0.54 and 0.55 mg kg^{-1} at 15 and 30 DAI respectively. At 90 DAI, $Zn_3(PO_4)_2$ recorded highest value of 0.58 mg kg^{-1}.Comparing different levels ofZn, application of 7.5 kg Zn ha^{-1} showed the highest value (0.61, 0.61, 0.63 and 0.64 mg kg^{-1}) whereas the lowest value was noticed in the treatment with no Zn application (0.39, 0.42, 0.47 and 0.49 mg kg^{-1}) in all the four stages of sampling. There was no significant difference in the WS-Ex fraction of Zn on ZSB application at 15 DAI whereas at 30, 60 and 90 DAI a significant increase in the WS-Ex fraction of Zn (0.55, 0.59 and 0.60 mg kg^{-1} respectively) as compared to the treatments without ZSB inoculation was noted (Table 2a).

Among the interactions, different sources of Zn along with ZSB had a significant influence on WS-Ex. fraction of Zn at 30, 60 and 90 DAI whereas the difference was not significant at 15 DAI. The highest mean value was obtained when $ZnSO_4$ was applied along with ZSB (0.57, 0.60 and 0.61 mg kg^{-1} at 30, 60 and 90 DAI respectively). Comparing the different combination of sources along with levels of Zn, significant difference was seen at 15 and 90 DAI but the variation observed at 30 and 60 DAI was not significant. At 15 DAI, the highest value was observed in the treatment with 7.5 kg Zn ha^{-1} as $ZnSO_4$ (0.65 mg kg^{-1}) whereas at 90 DAI, the highest value was observed in the treatment 7.5 kg Zn ha^{-1} as $Zn_3(PO_4)_2$ (0.66 mg kg^{-1}). When ZSB was applied along with different levels of Zn, a significant difference was noticed at 30, 60 and 90 DAI but not at 15 DAI, the highest value being observed when Zn was applied at the rate of 7.5 kg ha^{-1} along with ZSB (0.65, 0.67, 0.70 mg kg^{-1}respectively). Among the treatment combination containing sources, levels of Zn and ZSB, significant difference was recorded at 90 DAI with the highest value being observed when 7.5 kg Zn ha^{-1} was applied as $ZnSO_4$ along with ZSB (0.71 mg kg^{-1}) which was on par with the treatment 7.5 kg Zn ha^{-1} as $Zn_3(PO_4)_2$ along with ZSB (0.68 mg kg^{-1}).

Organically bound Zn (Org. Zn)

A significant difference in organically bound Zn was noticed on application of different levels of Zn (Table 3). The results indicated that application of 7.5 kg Zn ha^{-1} showed the highest mean value for Org. Zn (1.71, 1.73, 1.76 and 1.77 mg kg^{-1}respectively at 15, 30, 60 and 90 DAI). ZSB application had a significant influence on the Org. Zn (1.66, 1.68 and 1.70 mg kg^{-1}) compared to the treatment without the addition of ZSB (1.61, 1.64 and 1.65 mg kg^{-1}) at 30, 60 and 90 DAI, whereas at 15 DAI, non-significant difference was noticed (Table 3a). A significant effect was noticed in Org. Zn with application of different sources of Zn only at 15 DAI beyond which the variation was found to be non-significant.

Carbonate bound Zn (Car. Zn)

The results indicated a significant difference in carbonate bound Zn with the application of different levels of Zn at 15, 30, 60 and 90 DAI (Table 4). The highest value was observed when Zn was applied at the rate of 7.5 kg ha^{-1}(6.16, 6.18, 6.11 and 6.10 mg kg^{-1}) which was on par with 5 kg Zn ha^{-1} (6.00, 5.98, 5.94 and 5.86 mg kg^{-1}) at 15, 30, 60 and 90 DAI. Significant difference in carbonate bound Zn was observed with the application of ZSB at 60 and 90 DAI (Table 4a). Lowest Car. Zn was registered with ZSB application (mean values of 5.67 and 5.53 mg kg^{-1}respectively)at 60 and 90 DAI. Among the different sources, non-significant difference was noticed, at all stages of sampling. The interaction failed to attain the level of significance at all stages of sampling.

Fe-Mn oxide bound Zn (Fe-Mn Ox. Zn)

Different levels of Zn application was found to have significant effect on Fe-Mn oxide bound Zn, with the highest mean value beingreported at 7.5 kg Zn ha^{-1} (7.09, 7.23, 7.34 mg kg^{-1} respectively) which was on par with 5 kg Zn ha^{-1} (6.90, 7.17 and 7.25 mg kg^{-1} respectively) at 15, 30 and 90 DAI (Table 5). ZSB application failed to show significant influence on the Fe-Mn Ox. Zn at 15 and 30 DAI, whereas at 60 and 90 DAI, significant difference was noticed (Table 5a). Sources of Zn did not show any significant influence on Fe-Mn Ox. Zn at 15, 30 and 90 DAI whereas at 60 DAI application of $Zn_3(PO_4)_2$ recorded significantly highestFe-Mn Ox. Zn. Combination of different sources of Zn with ZSB had a significant influence on Fe-Mn oxide bound fraction of Zn at 90 DAI with the highest value beingrecorded when $ZnSO_4$ was used along with ZSB (7.46 mg kg^{-1}) which was on par with the application of $Zn_3(PO_4)_2$with ZSB (7.39 mg kg^{-1}). The interaction between different levels, sources and ZSB failed to exert significant influence on Fe-Mn Ox. Zn at different stages of sampling.

Residual Zn

Among the different levels of Zn, 7.5 kg Zn ha^{-1} recorded significantly highest residual Zn (84.5, 84.2, 84.2 and84.1 mg kg $^{-1}$) which was on par with 5 kg Zn ha^{-1} (83.9, 83.7, 83.5 and83.6 mg kg^{-1} respectively) at all stages of sampling (Table 6). No significant difference in the residual Zn was observed on application of different sources of Zn and ZSB (Table 6a). Interactions were found to have non-significant difference in the residual Zn.

Total Zn

Significant difference in total Zn was noticed with different levels of Zn at all the stages of sampling. Application of 7.5 kg Zn ha^{-1} (100, 100, 100 and 100 mg kg^{-1}) recorded highest total Zn which was on par with application of 5 kg Zn ha^{-1} (99.0, 99.1, 99 and 99.0mg kg^{-1}) respectively at all stages of sampling (Table 7). There was no significant difference noticed in total Zn with application of different sources of Zn and ZSB (Table 7a). Interactions failed to have significant influence on total Zn at all stages of sampling.

DISCUSSIONS

The distribution of Zn among different fractions was studied in calcareous soil through sequential extraction. Distribution of Zn fractions in the initial soil followed the order WS-Ex < organically bound < carbonate bound <Fe-Mn oxide bound< residual Zn. WS-Ex.Zn which represents the most readily available fraction, was found to be the lowest among different fractions (0.39% of total Zn). The high pH and high buffering capacity of the soil might be the reason for low availability of WS-Ex Zn (Deb, 1997 ;Lindsay, 1979 ;Xiang *et al.*, 1995 and Preetha and Stalin, 2014). A similar finding was also given by Wijebandara (2007)who reported that unfavourable pH and the presence of $CaCO_3$ in soils are responsible for the lower values of water soluble Zn fractions, which results in its easy precipitation.

Next to WS-Ex. Zn, organically bound Zn represents the lowest fraction (1.54% of total Zn). Residual fraction was found to be the highest (85.9% of total Zn) followed by Fe-Mn oxide bound Zn (6.60% of total Zn) and carbonate bound Zn (5.52% of the total Zn). It has been reported from the previous study that in calcareous soils, Zn fertilizer is first immobilized by soil organic matter and carbonates and then transformed into unavailable forms such as Min-Zn (Keshari, 2016).

The proportion of Fe-Mn oxide bound fraction of Zn was next to residual fraction. This might be due to the higher adsorption of Zn on the surfaces of oxides (Wijebandara, 2007). High amount of carbonate bound fraction of Zn is due to the presence of high $CaCO_3$ in calcareous soil. Among all the fractions, residual Zn was found to be the highest fraction which is in agreement with the findings from Fathi *et al.*(2014).

Among the sources of Zn, $ZnSO_4$ recorded higher values of WS-Ex Zn and Org. Zn during the initial stages of incubation. In the later stages of incubation (after 60 DAI), $Zn_3(PO_4)_2$ recorded higher values of these fractions. Readily soluble nature of $ZnSO_4$ and sparingly soluble nature of $Zn_3(PO_4)_2$ might be the reason for this trend. Increasing levels of Zn significantly increased all the fractions of Zn. Similar results were reported by Kamali*et al.*(2010).

With ZSB application, higher values of WS-Ex. Zn, Org. Zn, Fe-Mn Ox. Zn and lower values of Car. Zn were recorded when compared to the treatments without ZSB application. The variation noticed was significant after 30 DAI. The increase in the WS-Ex. Zn and Org. Zn fractions might be due to mineralization from recalcitrant sources such as carbonates and hydroxy- carbonates of Zn (Ramesh*et al.*, 2014). Reduction in soil pH as a result of increased microbial metabolic processes possibly increased the plant available fractions (Neumann and Romheld, 2003;Oburger*et al.*, 2009).Wide range of surface properties and Zn sorption characteristics of Fe and Mn oxides and the redistribution among different fractions of Zn with ZSB inoculation might be the reason for increase in Fe-Mn Ox. Zn (Zachara*et al.*, 1992)

The average percentage recovery of applied Zn into various fractions with ZSB inoculation were 5.30%, 5.71%, 6.11%, 29.1% and 52.9% respectively for WS-Ex. Zn, Org. Zn, Car. Zn, Fe-Mn Ox. Zn and residual Zn respectively. Whereas, without ZSB inoculation the average percentage recovery of applied Zn into different fractions were 3.90%, 3.91%, 17.2%, 18.6% and 53.2% respectively for WS-Ex. Zn, Org. Zn, Car. Zn, Fe-Mn Ox. Zn and residual Zn respectively. With ZSB inoculation, improved microbial activity in the soil resulted in depletion of Car. Zn and increase in WS-Ex. Zn, Org.Zn and Fe-Mn Ox. Zn and thereby increased the plant available Zn fractions (Ramesh*et al.*, 2014).

With advancement of incubation, increase in WS-Ex. Zn, Fe-Mn Ox. Zn, Org. Zn and decrease in Car. Zn were noticed with ZSB inoculation. In contrast, in the treatments which received $ZnSO_4$ which is a readily soluble Zn fertilizer and without ZSB inoculation, continuous reduction in WS-Ex. Zn, Org. Zn and Fe-Mn Ox. Zn and increase in Car. Zn were observed. The results indicated that in calcareous soils, major portion of applied Zn is converted into Car. Zn and the inoculation of ZSB helps in solubilizing the Car. Zn. Even without ZSB inoculation, $Zn_3(PO_4)_2$ caused slight increase in the plant available fractions of Zn with advancement of time. This might be due to the sparingly soluble nature of $Zn_3(PO_4)_2$ which enables supply of available Zn over extended period of time. Non- significant variation was observed in residual Zn over time.

CONCLUSIONS

The incubation study was conducted to study the transformation of Zn in calcareous soil. The results indicated that distribution of Zn fractions in the experimental soil is in the order WS-Ex. Zn < Org. Zn < Car. Zn < Fe-Mn Ox. Zn < residual Zn. WS-Ex. Zn representing the most readily available fraction is the lowest and residual Zn is the highest.During the initial stages of incubation, WS-Ex. Zn and Org. Zn were highest with $ZnSO_4$ application whereas during later stages of incubation higher values of these fractions were observed with $Zn_3(PO_4)_2$ application. Significant increase in all the fractions of Zn was observed with increasing levels of Zn. With ZSB inoculation, depletion of carbonate Zn and increase in WS-Ex. Zn, Org. Zn and Fe-Mn Ox. Zn was observed. Sources of Zn and ZSB application did not have significant influence on residual and total Zn. With increasing levels of Zn significant increase in all the Zn fractions and total Zn were observed and the highest value being recorded with 7.5 kg Zn ha^{-1}.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) here by declares that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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Table 2. Effect of sources, levels of Zn and ZSB on water soluble and exchangeable Zn in soil at different stages of incubation

Sources of Zn	Levels of Zn (kg ha^{-1})	Water soluble and exchangeable Zn (mg kg^{-1})															
		15 DAI				**30 DAI**				**60 DAI**				**90 DAI**			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	0.39	0.60	0.66	0.55	0.43	0.61	0.67	0.57	0.49	0.62	0.68	0.60	0.50	0.62	0.71	0.61
	(-) ZSB	0.38	0.59	0.64	0.54	0.40	0.57	0.59	0.52	0.46	0.53	0.57	0.52	0.48	0.51	0.53	0.51
	Mean	0.39	0.60	0.65	0.54	0.41	0.59	0.63	0.55	0.48	0.58	0.62	0.56	0.49	0.57	0.62	0.56
$Zn_3(PO_4)_2$	(+) ZSB	0.39	0.56	0.58	0.51	0.42	0.57	0.62	0.54	0.47	0.59	0.66	0.57	0.50	0.61	0.68	0.60
	(-) ZSB	0.38	0.54	0.56	0.49	0.42	0.56	0.58	0.52	0.47	0.58	0.62	0.56	0.48	0.59	0.64	0.57
	Mean	0.38	0.55	0.57	0.50	0.42	0.57	0.60	0.53	0.47	0.59	0.64	0.57	0.49	0.60	0.66	0.58
	Grand mean	0.39	0.58	0.61	0.52	0.42	0.58	0.61	0.54	0.47	0.58	0.63	0.56	0.49	0.58	0.64	0.57
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.01		0.01		0.01		0.02		0.01		NS		0.01		0.02	
	Z	0.01		NS		0.01		0.02		0.01		0.02		0.01		0.02	
	L	0.01		0.01		0.01		0.02		0.01		0.02		0.01		0.02	
	SZ	0.01		NS		0.01		0.02		0.01		0.02		0.01		0.02	
	ZL	0.01		NS		0.01		0.03		0.01		0.03		0.01		0.03	
	SL	0.01		0.03		0.01		NS		0.01		NS		0.01		0.03	
	SZL	0.01		NS		0.02		NS		0.02		NS		0.02		0.04	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 2a.Mean water soluble and exchangeable Zn at different stages of incubation for ZSB application.

ZSB / DAI	Water soluble and exchangeable Zn (mg kg^{-1})			
	15 DAI	**30 DAI**	**60 DAI**	**90 DAI**
(+) ZSB	0.53	0.55	0.59	0.60
(-) ZSB	0.52	0.52	0.54	0.54

Table 3. Effect of sources, levels of Zn and ZSB on organically bound Zn in soil at different stages of incubation

Sources of Zn	Levels of Zn ($kg\ ha^{-1}$)	Organically bound Zn ($mg\ kg^{-1}$)															
		15 DAI				30 DAI				60 DAI				90 DAI			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	1.49	1.71	1.78	1.66	1.51	1.72	1.80	1.67	1.56	1.73	1.81	1.70	1.59	1.73	1.82	1.71
	(-) ZSB	1.45	1.70	1.76	1.63	1.47	1.69	1.74	1.63	1.53	1.67	1.73	1.64	1.55	1.64	1.71	1.63
	Mean	1.47	1.71	1.77	1.65	1.49	1.70	1.77	1.65	1.55	1.70	1.77	1.67	1.57	1.68	1.77	1.67
$Zn_3(PO_4)_2$	(+) ZSB	1.48	1.65	1.69	1.61	1.52	1.68	1.72	1.64	1.55	1.69	1.76	1.67	1.58	1.71	1.78	1.69
	(-) ZSB	1.44	1.59	1.63	1.55	1.48	1.61	1.66	1.58	1.52	1.65	1.72	1.63	1.56	1.69	1.77	1.67
	Mean	1.46	1.62	1.66	1.58	1.50	1.64	1.69	1.61	1.54	1.67	1.74	1.65	1.57	1.70	1.77	1.68
	Grand mean	1.46	1.66	1.71	1.61	1.49	1.67	1.73	1.63	1.54	1.69	1.76	1.66	1.57	1.69	1.77	1.68
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.02		0.04		0.02		NS		0.02		NS		0.02		NS	
	Z	0.02		NS		0.02		0.04		0.02		0.05		0.02		0.05	
	L	0.03		0.05		0.03		0.05		0.03		0.06		0.03		0.06	
	SZ	0.03		NS		0.03		NS		0.03		NS		0.03		NS	
	ZL	0.04		NS		0.04		NS		0.04		NS		0.04		NS	
	SL	0.04		NS		0.04		NS		0.04		NS		0.04		NS	
	SZL	0.05		NS		0.05		NS		0.05		NS		0.05		NS	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 3a. Mean organically bound Zn at different stages of incubation for ZSB application

ZSB \ DAI	Organically bound Zn ($mg\ kg^{-1}$)			
	15 DAI	**30 DAI**	**60 DAI**	**90 DAI**
(+) ZSB	1.63	1.66	1.68	1.70
(-) ZSB	1.59	1.61	1.64	1.65

Table 4. Effect of sources, levels of Zn and ZSB on carbonate-bound Zn in soil at different stages of incubation

Sources of Zn	Levels of Zn (kg ha^{-1})	Carbonate- bound Zn (mg kg^{-1})															
		15 DAI				30 DAI				60 DAI				90 DAI			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	5.71	6.08	6.24	6.01	5.44	5.95	6.20	5.87	5.32	5.82	5.94	5.70	5.24	5.61	5.82	5.55
	(-) ZSB	5.77	6.01	6.18	5.99	5.60	6.08	6.22	5.97	5.55	6.15	6.35	6.02	5.49	6.16	6.44	6.03
	Mean	5.74	6.05	6.21	6.00	5.52	6.02	6.21	5.92	5.44	5.99	6.15	5.86	5.36	5.88	6.13	5.79
$Zn_3(PO_4)_2$	(+) ZSB	5.73	5.97	6.13	5.94	5.41	5.91	6.12	5.81	5.36	5.75	5.81	5.64	5.23	5.52	5.75	5.50
	(-) ZSB	5.75	5.92	6.08	5.92	5.63	5.96	6.18	5.93	5.53	6.03	6.31	5.96	5.47	6.15	6.39	6.00
	Mean	5.74	5.95	6.10	5.93	5.52	5.94	6.15	5.87	5.45	5.89	6.06	5.80	5.35	5.83	6.07	5.75
	Grand mean	5.74	6.00	6.16	5.97	5.52	5.98	6.18	5.89	5.44	5.94	6.11	5.83	5.35	5.86	6.10	5.77
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.08		NS		0.08		NS		0.07		NS		0.08		NS	
	Z	0.08		NS		0.08		NS		0.07		0.15		0.08		0.16	
	L	0.10		0.20		0.09		0.19		0.09		0.19		0.09		0.19	
	SZ	0.11		NS		0.11		NS		0.11		NS		0.11		NS	
	ZL	0.14		NS		0.13		NS		0.13		NS		0.13		NS	
	SL	0.14		NS		0.13		NS		0.13		NS		0.13		NS	
	SZL	0.19		NS		0.18		NS		0.18		NS		0.18		NS	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 4a. Mean carbonate-bound Zn in soil at different stages of incubation for ZSB application

ZSB / DAI	Carbonate- bound Zn (mg kg^{-1})			
	15 DAI	30 DAI	60 DAI	90 DAI
(+) ZSB	5.98	5.84	5.67	5.53
(-) ZSB	5.96	5.95	5.99	6.02

Table 5.Effect of sources, levels of Zn and ZSB on Fe-Mn oxide bound Zn in soil at different stages of incubation.

Sources of Zn	Levels of Zn (kg ha^{-1})	Fe-Mn oxide bound Zn (mg kg^{-1})															
		15 DAI				30 DAI				60 DAI				90 DAI			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	6.77	6.93	7.00	6.90	7.08	7.18	7.30	7.19	7.12	7.36	7.35	7.28	7.10	7.59	7.68	7.46
	(-) ZSB	6.88	6.96	7.08	6.97	7.00	7.07	7.27	7.11	7.02	6.84	6.85	6.90	6.85	6.66	6.82	6.78
	Mean	6.83	6.94	7.04	6.94	7.04	7.13	7.28	7.15	7.07	7.10	7.10	7.09	6.98	7.13	7.25	7.12
$Zn_3(PO_4)_2$	(+) ZSB	6.77	6.81	7.11	6.90	6.98	7.22	7.14	7.11	7.06	7.35	7.62	7.34	7.13	7.46	7.57	7.39
	(-) ZSB	6.84	6.88	7.17	6.96	6.73	7.22	7.22	7.06	6.98	7.31	7.40	7.23	6.88	7.30	7.31	7.16
	Mean	6.81	6.85	7.14	6.93	6.86	7.22	7.18	7.09	7.02	7.33	7.51	7.28	7.01	7.38	7.44	7.28
	Grand mean	6.82	6.90	7.09	6.93	6.95	7.17	7.23	7.12	7.04	7.21	7.30	7.19	6.99	7.25	7.34	7.20
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.07		NS		0.09		NS		0.09		0.19		0.09		NS	
	Z	0.07		NS		0.09		NS		0.09		0.19		0.09		0.19	
	L	0.08		0.17		0.11		0.23		0.11		NS		0.11		0.24	
	SZ	0.09		NS		0.13		NS		0.13		NS		0.13		0.27	
	ZL	0.12		NS		0.16		NS		0.16		NS		0.16		NS	
	SL	0.12		NS		0.16		NS		0.16		NS		0.16		NS	
	SZL	0.17		NS		0.23		NS		0.23		NS		0.23		NS	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 5a. Mean Fe-Mn oxide bound Zn in soil at different stages of incubation for ZSB application

ZSB / DAI	Fe-Mn oxide bound Zn (mg kg^{-1})			
	15 DAI	30 DAI	60 DAI	90 DAI
(+) ZSB	6.90	7.15	7.31	7.42
(-) ZSB	6.97	7.09	7.06	6.97

Table 6. Effect of sources, levels of Zn and ZSB on residual Zn at different stages of incubation

Sources of Zn	Levels of Zn (kg ha^{-1})	Residual Zn (mg kg^{-1})															
		15 DAI				30 DAI				60 DAI				90 DAI			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	82.4	83.8	84.3	83.5	82.4	83.5	84.1	83.4	82.4	83.4	84.1	83.3	82.4	83.6	84.1	83.3
	(-) ZSB	82.3	83.7	84.4	83.5	82.4	83.7	84.2	83.4	82.3	83.8	84.4	83.5	82.4	83.9	84.5	83.6
	Mean	82.4	83.8	84.4	83.5	82.4	83.6	84.2	83.4	82.4	83.6	84.2	83.4	82.4	83.7	84.3	83.5
$Zn_3(PO_4)_2$	(+) ZSB	82.5	84.0	84.6	83.7	82.5	83.7	84.3	83.5	82.4	83.5	84.3	83.4	82.5	83.7	84.2	83.5
	(-) ZSB	82.5	84.0	84.7	83.7	82.4	83.7	84.2	83.5	82.4	83.4	84.0	83.3	82.4	83.4	83.8	83.2
	Mean	82.5	84.0	84.6	83.7	82.5	83.7	84.2	83.5	82.4	83.5	84.1	83.3	82.4	83.5	84.0	83.3
	Grand mean	82.4	83.9	84.5	83.6	82.5	83.7	84.2	83.4	82.4	83.5	84.2	83.4	82.4	83.6	84.1	83.4
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.5		NS		0.5		NS		0.5		NS		0.5		NS	
	Z	0.5		NS		0.5		NS		0.5		NS		0.5		NS	
	L	0.6		1.3		0.6		1.3		0.6		1.3		0.6		1.3	
	SZ	0.7		NS		0.7		NS		0.7		NS		0.7		NS	
	ZL	0.9		NS		0.9		NS		0.9		NS		0.9		NS	
	SL	0.9		NS		0.9		NS		0.9		NS		0.9		NS	
	SZL	1.2		NS		1.2		NS		1.2		NS		1.2		NS	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 6a. Mean residual Zn at different stages of incubation for ZSB application

ZSB / DAI	Residual Zn (mg kg^{-1})			
	15 DAI	**30 DAI**	**60 DAI**	**90 DAI**
(+) ZSB	83.6	83.4	83.3	83.4
(-) ZSB	83.6	83.4	83.4	83.4

Table 7. Effect of sources, levels of Zn and ZSB on total Zn at different stages of incubation

Levels of Zn (kg ha^{-1}) / Sources of Zn		Total Zn (mg kg^{-1})															
		15 DAI				30 DAI				60 DAI				90 DAI			
		0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean	0	5.0	7.5	Mean
$ZnSO_4$	(+) ZSB	96.8	99.1	100	98.6	96.9	99.0	100	98.7	96.9	98.9	99.9	98.6	96.8	99.1	100	98.7
	(-) ZSB	96.8	99.0	100	98.6	96.9	99.1	100	98.7	96.9	99.0	99.9	98.6	96.8	98.9	100	98.6
	Mean	96.8	99.1	100	98.6	96.9	99.1	100	98.7	96.9	99.0	99.9	98.6	96.8	99.0	100	98.6
$Zn_3(PO_4)_2$	(+) ZSB	96.9	99.0	100	98.7	96.8	99.1	99.9	98.6	96.8	98.9	100	98.6	96.9	99.0	100	98.6
	(-) ZSB	96.9	98.9	100	98.6	96.7	99.1	99.8	98.5	96.9	99.0	100	98.6	96.8	99.1	99.9	98.6
	Mean	96.9	99.0	100	98.7	96.8	99.1	99.8	98.5	96.9	99.0	100	98.6	96.9	99.1	100	98.6
	Grand mean	96.9	99.0	100	98.6	96.8	99.1	100	98.6	96.9	99.0	100	98.6	96.8	99.0	100	98.6
		SEd		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**		**SEd**		**CD (P=0.05)**	
	S	0.68		NS		0.70		NS		0.69		NS		0.70		NS	
	Z	0.68		NS		0.70		NS		0.69		NS		0.70		NS	
	L	0.83		1.71		0.85		1.76		0.85		1.75		0.86		1.77	
	SZ	0.96		NS		0.99		NS		0.98		NS		0.99		NS	
	ZL	1.17		NS		1.21		NS		1.20		NS		1.21		NS	
	SL	1.17		NS		1.21		NS		1.20		NS		1.21		NS	
	SZL	1.66		NS		1.71		NS		1.70		NS		1.72		NS	

*S – Sources of Zn; Z – Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 7a. Mean total Zn at different stages of incubation for ZSB application

ZSB / DAI	Total Zn (mg kg^{-1})			
	15 DAI	**30 DAI**	**60 DAI**	**90 DAI**
(+) ZSB	98.7	98.6	98.6	98.7
(-) ZSB	98.6	98.6	98.6	98.6