

Zinc availability in calcareous soil as influenced by various levels, sources of Zn and Zn solubilizing bacteria

Abstract

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. Microbial transformation of Zn from unavailable form to soil available form is an important approach contributing to plant Zn nutrition. An incubation experiment was conducted to study the transformation of Zn in calcareous soil. The results of incubation experiment revealed that application of Zn fertilizers with Zinc Solubilizing Bacteria (ZSB) reduced the soil pH, increased the EC and DTPA-Zn content of the soil. It was found that ZnSO₄ addition at 7.5 kg Zn ha⁻¹ along with ZSB (*Enterobacter cloacae*) was considered to be the best Zn management strategy in calcareous soil for sustaining Zn availability to crops..

Key words: Calcareous soil, Maize, pH, EC and DTPA Zn, Zinc Solubilizing Bacteria

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. Zinc is involved in different functions in plants including major roles in enzyme reaction, DNA transcription and auxin activity. Zn is important in 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). Zinc deficiency results in stunted growth of the plants, decreasing the number of tillers, chlorosis and smaller leaves, delayed crop maturity, spikelet sterility and inferior quality of harvested products (Hafeez *et al.*, 2013).

Deficiency of Zn appears to be the most widespread problem in crops and pasture plants worldwide, resulting in severe loss in yield and nutritional quality. Analysis of 24,2827 surface soil samples (0 to 15 cm depth) from the agriculture fields of 615 districts in 28 states of India revealed that around 51.2 per cent of Indian soils are Zn deficient (Shukla, 2021). Zn deficiency 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 acid soils, which are leached and heavily weathered (Alloway, 2004; Behera *et al.*, 2011). Due to prevalent Zn deficiency, the production of cereal crops suffers leading to the twin problem of low food production and Zn malnutrition in the population which uses cereals as their staple diet. According to a report published by WHO, deficiency of Zn was considered as the fifth most important risk factor responsible for illness and death in the developing world (Cakmak, 2008).

Maize, known as the 'Queen of cereals' occupies third position in production next to wheat and rice in the world. Besides providing nutrients for human beings and animals, it also serves as a raw material for the production of oil, starch, protein, alcoholic beverages and food sweeteners. In India, maize is grown in a wide range of environment, extending from extreme semi-arid to sub humid and humid regions. It is grown in an area of 9,63,320 hectare with a production of about 25.89 million tonnes and productivity of 2689 kg ha⁻¹ in India. In Tamil Nadu, it is grown in an area of 31,503 hectares with a production of 0.95 million tonnes (Indiastat., 2017).

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 CaCO₃ 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.

Calcareous soil occupies about 800 mha of area globally. 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).

Zinc sulphate, a common soluble form of Zn when applied to calcareous soil may get converted to insoluble form of Zn (Eq. 1.1) (Sadiq, 1991). Since zinc solubilizing bacteria (ZSB) have a great potential to solubilize the insoluble Zn sources like ZnCO₃, ZnO, Zn₃(PO₄)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. Zinc solubilizing bacteria are those bacteria which are capable of solubilizing the insoluble Zn compounds or minerals in agar plate as well as in soil (Sarathambal *et al.*, 2010). ZSB helps in acquiring Zn from Zn deficient soil as compared to un-inoculated plants. The bacteria solubilize Zn through various mechanism, which include excretion of metabolites such as organic acids, proton extrusion or production of chelating agents (Fasim *et al.*, 2002). Gluconic acid produced by bacteria helps in solubilization of zinc salts and increased growth of maize (Goteti *et al.*, 2013). Bacteria isolated from Zn deficient soil has Zn solubilizing activity by lowering down the pH due to gluconic acid production and were also positive for indole acetic acid production (Vaid *et al.*, 2014). This solubilisation property is important in calcareous soils which are Zn deficient.

As unavailability of Zn is an important limiting factor in calcareous soils, it is imperative to evaluate the response of Zn nutrition on maize productivity. Also information on the influence of ZSB along with Zn levels and sources on the management of Zn in calcareous soil are still minimum. Hence the present study was undertaken to determine the effect of ZSB (*Enterobacter cloacae*) inoculation with different levels, sources of Zn on the growth, yield and zinc uptake by maize grown in calcareous soil.

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 (Strain ZSB14). The experiment was conducted in black calcareous soil collected from Eastern block, TNAU Coimbatore with two sources (ZnSO_4 and $\text{Zn}_3(\text{PO}_4)_2$), 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 $\text{Zn}_3(\text{PO}_4)_2$ 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 10⁸ 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 pH, electrical conductivity, free CaCO₃, DTPA Zn as per the procedures outlined in Table 1.

Table 1. Analytical methods used in soil sample analysis

Parameters	Methodology	Reference
Physical Properties		
Particle size analysis	International Pipette method	Piper (1966)
Physico - chemical Properties		
Soil reaction (pH)	Potentiometry(1:2.5 soil: water suspension)	Jackson (1973)
Electrical conductivity	Conductometry(1:2.5 soil : water suspension)	Jackson (1973)
Free CaCO ₃	Rapid titration method	Piper (1931)
Chemical Properties		
Soil Organic Carbon	Chromic acid wet digestion method	Walkleyand Black (1934)
Cation Exchange Capacity	Neutral N NH ₄ OAc	Jackson (1973)
Available Nitrogen (N)	Alkaline permanganate method	Subbiah and Asija(1956)
Available Phosphorus (P)	Olsen method	Olsen (1954)
Available Potassium (K)	Neutral N NH ₄ OAc	Stanford and English (1949)
Exchangeable Calcium and Magnesium	Versenate titration (Neutral N NH ₄ OAc extract)	Jackson (1973)

DTPA Zn ,Cu, Fe and Mn	Atomic Absorption Spectrophotometer	Lindsay and Norvell(1978)
------------------------	-------------------------------------	---------------------------

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 and Discussion

Composite surface soil sample was collected from Eastern Block, TNAU, Coimbatore for conducting pot culture and incubation experiments. The experimental soil belongs to Periyanaickenpalayam series and comes under the taxonomic classification fine, montmorillonitic, isohyperthermic, calcareous Typic Haplustert. The soil texture was clay loam. The soil was alkaline in reaction (8.47) with permissible amount of soluble salts (0.31 dS m⁻¹). The experimental soil was moderately calcareous with a free CaCO₃ content of 12.0%. The organic carbon content of the soil was medium (5.4 g kg⁻¹) (Medium:5.0 to 7.5 g kg⁻¹). The CEC of the soil was 44.1 C mol (p⁺) kg⁻¹.

The soil was low in available N (232 kg ha⁻¹), medium in available P (17.9 kg ha⁻¹) and high in available K (588 kg ha⁻¹). The exchangeable Ca and Mg content of the soil were 32.5 and 10.4 C mol (p⁺) kg⁻¹ respectively. The soil was deficient in DTPA-Zn (0.59 mg kg⁻¹), DTPA-Fe (2.11 mg kg⁻¹), DTPA-Cu (0.79 mg kg⁻¹) and sufficient in DTPA-Mn (6.33 mg kg⁻¹) (Critical values followed in Tamil Nadu: Zn-1.2 mg kg⁻¹; Fe: Clacaroeous soil- 6.3 mg kg⁻¹; Cu-1.2 mg kg⁻¹; Mn- 2.0 mg kg⁻¹).

The incubation experiment was conducted to study the transformation of zinc in calcareous soil. Soil pH, EC and available Zn were recorded at different days after incubation (15, 30, 60, 90 DAI).The results obtained were given below.

Soil pH

Reduction in soil pH was noticed with advancement of incubation (Table 2). Application of different levels of Zn had significant influence on soil pH at all stages of sampling. With reference to different levels of Zn, application of Zn at 7.5 kg ha⁻¹

registered the lowest mean pH value of 8.19, 8.00, 7.91 and 7.79 respectively at 15, 30, 60 and 90 DAI. Zn unamended control registered the highest mean pH value of 8.35, 8.22, 8.17 and 8.13 respectively at 15, 30, 60 and 90 DAI. Zinc solubilizing bacteria application was found to have significant effect on soil pH at 30, 60 and 90 DAI. ZSB inoculation recorded significantly lowest pH of 8.04, 7.95 and 7.83 respectively at 30, 60 and 90 DAI (Table 2a). Significant variation was not observed with the application of different sources of Zn at all stages of sampling. However ZnSO₄ application registered lower pH when compared to Zn₃(PO₄)₂. The interaction effect of Zn sources, levels and Zn solubilizing bacteria was not significant at 15, 30 and 60 DAI. At 90 DAI, interaction between ZSB and levels of Zn was found to be significant. At 90 DAI, significantly lowest soil pH (7.67) was observed in the treatment which received ZnSO₄ at 7.5 kg Zn ha⁻¹ along with ZSB and the highest value (8.36) was observed in the treatment without Zn and ZSB application.

The reduction in the pH of the soil with the addition of ZSB may be due to excretion of organic acids and proton extrusion by microbial isolates might be the possible reason for the reduction in pH of the soil. Similar results were already reported by Goteti *et al.* (2013) and Ramesh *et al.* (2014). Among the different levels of Zn application, 7.5 kg Zn ha⁻¹ significantly reduced the pH compared to other levels which might be probably due to the additional supply of sulphur from ZnSO₄ with higher levels of Zn which was corroborated with the findings of Mahler and Maples (1986) and El-Badawy and Mehasen (2011). Higher levels of ZnSO₄ addition exerted complimentary effect in reducing soil pH.

Soil EC

With advancement of incubation, soil EC increased. The results indicated a significant difference in the EC of the soil with the application of different sources, levels of Zn and ZSB (Table 3). Among different sources, application of ZnSO₄ showed the highest mean EC value of 0.38, 0.41, 0.45 and 0.47 dS m⁻¹ respectively at 15, 30, 60, and 90 DAI. Comparison of different Zn levels indicated that, application of 7.5 kg Zn ha⁻¹ recorded the highest mean EC at 15, 30, 60, and 90 DAI (0.42, 0.46, 0.51 and 0.54 dS m⁻¹ respectively) and the lowest mean value was observed in the treatment without Zn application (0.33, 0.34, 0.36 and 0.36 dS m⁻¹ respectively). ZSB application also significantly increased the EC (0.38, 0.42, 0.45 and 0.48 dS m⁻¹ respectively) at 15, 30, 60 and 90 DAI as compared to the treatment without ZSB (Table 3a).

Among the interactions, significant differences were noticed between different sources and levels of Zn. The highest EC was observed in treatment where 7.5 kg ha⁻¹ Zn was applied in the form of ZnSO₄ (0.44, 0.47, 0.53 and 0.57 dSm⁻¹ respectively) and the lowest value at 15, 30, 60 and 90 DAI was observed in the treatment without Zn application.

An increase in the electrical conductivity of soil could be due to the soluble salts during the solubilization of Zn (Goteti *et al.*, 2013). ZnSO₄ application registered higher soil EC as compared to the application of Zn₃(PO₄)₂. This might be due to the effect of sulphuric acid formed during the dissolution of ZnSO₄ which undergoes reaction with CaCO₃ in the calcareous soils which leads to the formation of gypsum. Similar results were already reported by Kaplan and Orman (1998) and Heydarnezhad *et al.* (2012).

DTPA-Zn

A significant difference was seen on the available Zn content on application of different sources, levels of Zn and ZSB (Table 4). Application of 7.5 kg Zn ha⁻¹ as ZnSO₄ along with ZSB recorded the highest DTPA-Zn content (1.59, 1.65, 1.71, 1.78 mg kg⁻¹) whereas the lowest value of DTPA-Zn was observed in the treatment without Zn and ZSB application (0.75, 0.82, 0.85, 0.91 mg kg⁻¹) at 15, 30, 60, 90 DAI respectively. Among the different sources of Zn, a significant difference in Zn content was noticed at 15 and 30 DAI whereas the variation was not significant at 60 and 90 DAI. Zn application as ZnSO₄ registered the highest mean DTPA-Zn (1.24, 1.25 mg kg⁻¹) as compared to Zn₃(PO₄)₂ (1.06 and 1.15 mg kg⁻¹) at 15 and 30 DAI respectively. Different levels of Zn showed a significant difference in DTPA-Zn where the highest mean value was obtained in treatment which received 7.5 kg Zn ha⁻¹ (1.38, 1.40, 1.42 and 1.45 mg kg⁻¹) and the lowest value with no Zn application. ZSB application significantly increased the DTPA-Zn content of the soil. The highest mean DTPA-Zn was observed in the treatment with ZSB (1.24, 1.33, 1.41 and 1.47 mg kg⁻¹ at 15, 30, 60 and 90 DAI respectively) (Table 4a).

The interaction between different sources and levels of Zn showed significant difference in Zn content at 15 and 30 DAI with highest mean Zn content being recorded in the treatment which received 7.5 kg Zn ha⁻¹ as ZnSO₄ (1.52 and 1.48 mg kg⁻¹ respectively), whereas the lowest value being observed in the treatment without Zn (0.85 and 0.93 mg kg⁻¹). Sources of Zn along with ZSB showed a significant difference in DTPA-Zn content at 60 and 90 DAI with highest mean value being observed in the

treatment with ZnSO₄ along with ZSB (1.45 and 1.52 mg kg⁻¹ respectively). The treatment combination of different levels of Zn along with ZSB showed significant variation at 30, 60 and 90 DAI with the highest mean Zn content recorded in the treatment with 7.5 kg Zn ha⁻¹ along with ZSB (1.56, 1.65, 1.73 mg kg⁻¹ respectively). Application of different sources and levels of Zn along with ZSB also showed significant variation at 15 and 90 DAI whereas there was no significant difference at 30 and 60 DAI. The highest mean value of 1.59 and 1.78 mg kg⁻¹ on 15 and 90 DAI was reported when Zn was applied at the rate of 7.5 kg Zn ha⁻¹ in the form of ZnSO₄ along with ZSB.

As expected, increase in levels of Zn significantly increased the DTPA-Zn content of soil at all the stages. During the initial stages of incubation, ZnSO₄ registered significantly higher DTPA- Zn when compared to Zn₃(PO₄)₂ which became comparable at later stages. This might be due to the readily soluble nature of ZnSO₄ and sparingly soluble nature of Zn₃(PO₄)₂. Higher solubilization of insoluble Zn was achieved with the application of ZSB, which increased the available Zn content of the soil. The solubilization of Zn by ZSB might be achieved due to the production of different organic acids like gluconic acid and due to proton extrusion and production of chelating agents as suggested by Krithika *et al.*(2016) and Anuradha *et al.* (2015). Improved DTPA- Zn content of the soil through production of organic acids and enhanced microbial activity was also reported by Ramesh *et al.* (2014) and Shakeel *et al.*(2015). Di Simine *et al.*(1998) investigated the solubilization of Zn₃(PO₄)₂ by a strain of *Pseudomonas fluorescense* and found that gluconic acids helped in solubilization of Zn salts. Negative and significant correlation was observed between pH and DTPA-Zn (-0.549**).

SUMMARY AND CONCLUSION

The results indicated that, with advancement of incubation, reduction in soil pH was observed. Though ZnSO₄ application was not significant on soil pH, it reduced the soil pH during the incubation period when compared to Zn₃(PO₄)₂. With increase in levels of Zn, pH was reduced and application of 7.5 kg ha⁻¹ recorded significantly lower mean pH value. With ZSB application lowest mean pH values were recorded when compared to the treatments without ZSB application. Increase in soil EC was observed with advancement of incubation. Among the sources, application of ZnSO₄ and among the levels, application of 7.5 kg Zn ha⁻¹ registered significantly higher EC values. When compared to the treatments without ZSB application, significantly higher EC values were recorded in the treatments with ZSB application. During the initial stages, significantly higher DTPA-Zn

was observed with ZnSO₄ application over Zn₃(PO₄)₂ which became comparable at later stages. With increasing levels of Zn, significant increase in soil DTPA-Zn content was noticed at all stages of sampling and the highest Zn content being recorded with 7.5 kg Zn ha⁻¹. ZSB application registered significantly higher soil DTPA-Zn content.

It could be concluded that ZnSO₄ addition at 7.5 kg Zn ha⁻¹ along with ZSB (*Enterobacter cloacae*) was considered to be the best Zn management strategy in calcareous soil for obtaining higher yield of maize crop and sustaining soil fertility.

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.

REFERENCES

- Alloway, B.J. . Zinc in soils and crop nutrition: International Zinc Association Brussels. 2004; Pp. 135.
- Anuradha, P., Syed I., Swati , M. and Patil, V. Solubilisation of insoluble zinc compounds by different microbial isolates invitro condition. International Journal of Tropical Agriculture. 33(Part II), 2015; 865-869.
- Behera, S. Singh, M., Singh, K. and Todwal, S. Distribution variability of total and extractable zinc in cultivated acid soils of India and their relationship with some selected soil properties. Geoderma.2011; 162 (304), 242-250.
- Behera, S., Shukla, A.K., Singh, M., Wnajari, R.H. and Singh, P. Yield and Zinc, copper, manganese and iron concentration in maize (*Zea Mays L*) – Wheat (*Triticum aestivum L.*) grown on Vertisol as influenced by zinc application from various zinc fertilizers. Journal of plant nutrition. 2015; 38 (10), 1544-1557.
- Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic bio fortification. Plant and soils. 2008; 302 (1-2): 1-17.
- Di Simine, C., Sayer, J., and Gadd, G. Solubilisation of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soils. Biology and fertility of soils. 1998; 28(1): 87-94.
- El-Badawy, M.E.M. and Mehasen, S. Multivariate analysis for yield and its components in maize under zinc and nitrogen fertilization levels. Australian journal of Basic and Applied Sciences., 2011; 5(12):3008-3015.
- Fasim, F., Ahmed, N., Parsons, R. and Gadd, G. G.M.Solubilisation of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS microbiology letters. 2002; 213 (1):1-6).
- Goteti, P.K. Emmanuel, L.D.A., Desai, S. and Shaik, M.H.A. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea Mays L.*) International Journal of Microbiology.2013; 2013:1–7.
- Hafeez, B. Khanif, Y. and Saleem, M. Role of zinc in plant nutrition - a review. American journal of experimental Agriculture. 2013; 3(2): 374

- Heydarnezhad, F. Shahinrokhsar, P. and Shokri, V.H. Influence of elemental sulfur and sulfur oxidising Bacteria on some nutrient deficiency in calcareous soils. *International Journal of Agriculture and Crop Sciences*. 2012; 4(12):735-739.
- Hu, Y. Huang, Y., Su, J., Gao, Z., Li, S. and Nan, Z. Temporal changes of metal bioavailability and extracellular enzyme activities in relation to afforestation of highly contaminated calcareous soil. *Science of the Total environment*. 2018; 622: 1056-1066.
- Jackson, ML. *Methods of chemical analysis*. Prentice Hall., Englewood Cliffs, NJ. 1973. Pp 521
- Katyal, J. and Vlek, P. Micronutrient problems in tropical Asia. *Fertilizer Research*. 1985; 7(1-3):69-94.
- Kaplan, M. and Orman, S. Effect of elemental sulphur and sulphur containing waste in a calcareous soil in Turkey. *Journal of Plant Nutrition*. 1998; 21(8):1655-1665.
- Krithika, S. Prasad, G., and Balachandar, D. Zinc solubilizing potential of *Entirobacter cloacae* Strain ZSB14 in Three Different Semi-arid Tropical soils. *International Journal of Plant and Soil Science*. 2016; 11(2):1-12.
- Lindsay, WL and Norvell, WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 1978; 42 (3):421-428.
- Mahler, R.J. and Maples, R.L. Response of wheat to sulphur fertilisation. *Communications in Soil Science and Plant analysis*. 1986; 17(9): 975-988.
- Olsen, Sterling R. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*: Circular No. 939, United States Department of Agriculture, Washington, 1954; 1-22
- Panase, Vinayak Govind, Pandurang Vasudeo Sukhatme, and Frederick John Freshwater Shaw. *Statistical Methods for Agricultural Workers: By VG Panase and PV Sukhatme*: Indian Council of Agricultural Research. 1967; Pp.381.
- Piper, C. . "Soil and Plant Analysis 1944." *Soil and Plant Analysis*, 1931; Pp.294
- Piper, Clarence Sherwood. *Soil and plant analysis*: Hans Publishers; Bombay, 1966; Pp 368
- Ramesh, A. Sharma, S.K. Sharma, M.P., Yadav, N. and Joshi, O.P. Inoculation of zinc solubilizing *Bacillus aryabhatai* strains for improved growth, mobilisation and bio fortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied soil Ecology*. 2014; 73:87-96.
- Sadiq, M. Solubility and speciation of zinc in calareous soils. *Water, Air and Soil Pollution*. 1991; 57 (1):411-421.
- Sarathambal, C. Thangaraju, M. Paulraj, C. and Gomathy, M. Assessing the zinc solubilisation ability of *Gluconobacter daizotrophucus* in maize rhizosphere using labelled ⁶⁵Zn compounds. *Indian Journal of Microbiology*. 2010; 50(1): 103-109.
- Saravanan, V.S. Subramoniam, S.R., and Raj, S.A. Assessing invitro solubilisation of potential of different zinc solubilizing bacterial isolates. *Brazialin Journal of Microbiology*. 2004; 35(1-2):121-125.
- Sentimenla, Status of Zinc Availability in Jhum Fields under Rainfed Condition in Zunheboto District of Nagaland. *International Journal of Plant & Soil Science*, 2020; 32 (20):25-30. <https://doi.org/10.9734/ijpss/2020/v32i2030399>.

- Shakeel, M. Rais, A. Hassan, M.N. and Hafeez, F.Y. Root associated bacillus sp. Improves growth, yield and zinc application on maize (*Zea mays* L.). *International Journal of Agronomy and Agricultural Research*, 2015; 9 (3) : 66-75.
- Shukla, A.K., Behera, S.K., Prakash, C., Patra, A.K., Rao, C.S., Chaudhari, S.K., Das, S., Singh, A.K., Green, A. Assessing Multi-Micronutrients Deficiency in Agricultural Soils of India. *Sustainability*, 2021; 13, 9136:1-19.
<https://doi.org/10.3390/su13169136>
- Stanford, George, and Leah English. Use of the flame photometer in rapid soil tests for K and Ca. *Agronomy Journal*. 1949; 41 (9):446-447.
- Subbiah, BV, and Asija GL A rapid method for the estimation of nitrogen in soil. *Current Science*, 1956; 26:259-260.
- Vaid, S.K. Kumar, B., Sharma, A. Shukla, A.K. Srivastava, P. Effect of zinc solubilizing bacteria on growth and zinc nutrition of rice. *Journal of soil science and plant nutrition*. 2014; 14 (4):889-910.
- Walkley, Aldous, and Armstrong Black I. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 1934; 37 (1):29-38.
- Zhang, Y, Yan, Y. Fu, C., Li, M. and Wang, Y.A. Zinc sulphate spray increases activity of carbohydrate metabolic enzymes and regulates endogenous hormone levels in apple fruit. *Scientia Horticulturae*. 2016; 211: 363-368.

Table 2. Effect of sources, levels of Zn and ZSB on soil pH at different stages of incubation

Levels of Zn Sources of Zn (kg ha ⁻¹)		Soil pH															
		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	8.33	8.19	8.06	8.19	8.11	8.00	7.82	7.98	8.03	7.94	7.81	7.92	7.91	7.78	7.67	7.79
	(-) ZSB	8.39	8.30	8.18	8.29	8.32	8.13	7.99	8.15	8.31	8.04	7.92	8.09	8.32	7.94	7.79	8.02
	Mean	8.36	8.24	8.12	8.24	8.21	8.07	7.91	8.06	8.17	7.99	7.86	8.01	8.12	7.86	7.73	7.90
Zn ₃ (PO ₄) ₂	(+) ZSB	8.32	8.31	8.22	8.28	8.14	8.12	8.06	8.11	8.02	8.00	7.93	7.98	7.91	7.89	7.82	7.87
	(-) ZSB	8.38	8.34	8.29	8.34	8.33	8.13	8.11	8.19	8.35	8.05	7.99	8.13	8.36	7.92	7.88	8.05
	Mean	8.35	8.32	8.25	8.31	8.24	8.13	8.09	8.15	8.18	8.02	7.96	8.05	8.14	7.91	7.85	7.96
Grand mean		8.35	8.28	8.19	8.28	8.22	8.10	8.00	8.11	8.17	8.00	7.91	8.03	8.13	7.88	7.79	7.93
		SEd		CD (P=0.05)		SEd		CD (P=0.05)		SEd		CD (P=0.05)		SEd		CD (P=0.05)	
S		0.04		NS		0.05		NS		0.05		NS		0.05		NS	
Z		0.04		NS		0.05		0.11		0.05		0.09		0.05		0.10	
L		0.05		0.11		0.06		0.13		0.06		0.12		0.06		0.12	
SZ		0.06		NS		0.07		NS		0.06		NS		0.07		NS	
ZL		0.07		NS		0.09		NS		0.08		NS		0.09		0.17	
SL		0.07		NS		0.09		NS		0.08		NS		0.08		NS	
SZL		0.10		NS		0.12		NS		0.11		NS		0.12		NS	

*S – Sources of Zn; Z–Without Zinc Solubilising Bacteria (ZSB); L – Levels of Zn

Table 2a. Mean soil pH at different stages of incubation for ZSB application

ZSB DAI		Soil pH															
		15 DAI				30 DAI				60 DAI				90 DAI			
(+) ZSB		8.24				8.04				7.95				7.83			
(-) ZSB		8.31				8.17				8.11				8.04			

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

Sources of Zn Levels of Zn (kg ha ⁻¹)		Soil EC (dS m ⁻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	0.34	0.39	0.45	0.39	0.35	0.43	0.49	0.42	0.37	0.47	0.55	0.46	0.38	0.51	0.60	0.50
	(-) ZSB	0.32	0.37	0.42	0.37	0.33	0.41	0.46	0.40	0.34	0.46	0.50	0.43	0.34	0.49	0.53	0.45
	Mean	0.33	0.38	0.44	0.38	0.34	0.42	0.47	0.41	0.36	0.47	0.53	0.45	0.36	0.50	0.57	0.47
Zn ₃ (PO ₄) ₂	(+) ZSB	0.35	0.36	0.42	0.38	0.36	0.41	0.46	0.41	0.37	0.44	0.50	0.44	0.37	0.48	0.53	0.46
	(-) ZSB	0.33	0.35	0.40	0.36	0.34	0.38	0.45	0.39	0.35	0.42	0.48	0.42	0.35	0.44	0.49	0.43
	Mean	0.34	0.35	0.41	0.37	0.35	0.39	0.45	0.40	0.36	0.43	0.49	0.43	0.36	0.46	0.51	0.44
Grand Mean		0.33	0.37	0.42	0.37	0.34	0.41	0.46	0.41	0.36	0.45	0.51	0.44	0.36	0.48	0.54	0.46
		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		NS		0.01		0.01		0.01		0.01	
Z		0.01		0.01		0.01		0.01		0.01		0.01		0.01		0.01	
L		0.01		0.01		0.01		0.01		0.01		0.01		0.01		0.02	
SZ		0.01		NS		0.01		NS		0.01		NS		0.01		NS	
ZL		0.01		NS		0.01		NS		0.01		NS		0.01		NS	
SL		0.01		0.02		0.01		0.02		0.01		0.02		0.01		0.02	
SZL		0.01		NS		0.01		NS		0.01		NS		0.02		NS	

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

Table 3a. Mean soil EC values at different stages of incubation for ZSB application

ZSB DAI		Soil EC (dS m ⁻¹)															
		15 DAI				30 DAI				60 DAI				90 DAI			
(+) ZSB		0.38				0.42				0.45				0.48			

(-) ZSB	0.36	0.39	0.43	0.44
---------	------	------	------	------

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

Levels of Zn Sources Of Zn (kg ha ⁻¹)		DTPA-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	0.95	1.41	1.59	1.32	1.05	1.47	1.65	1.39	1.12	1.53	1.71	1.45	1.19	1.59	1.78	1.52
	(-) ZSB	0.75	1.31	1.45	1.17	0.82	1.21	1.32	1.11	0.85	1.08	1.15	1.03	0.91	0.98	1.05	0.98
	Mean	0.85	1.36	1.52	1.24	0.93	1.34	1.48	1.25	0.99	1.31	1.43	1.24	1.05	1.29	1.41	1.25
Zn ₃ (PO ₄) ₂	(+) ZSB	0.92	1.21	1.34	1.16	1.02	1.31	1.47	1.26	1.10	1.38	1.59	1.36	1.15	1.47	1.67	1.43
	(-) ZSB	0.78	0.96	1.13	0.96	0.85	1.08	1.18	1.04	0.91	1.11	1.22	1.08	0.93	1.18	1.30	1.14
	Mean	0.85	1.08	1.23	1.06	0.94	1.19	1.33	1.15	1.01	1.25	1.40	1.22	1.04	1.32	1.48	1.28
Grand Mean		0.85	1.22	1.38	1.15	0.93	1.27	1.40	1.20	1.00	1.27	1.42	1.23	1.04	1.31	1.45	1.27
		SEd		CD (P=0.05)		SEd		CD (P=0.05)		SEd		CD (P=0.05)		SEd		CD (P=0.05)	
S		0.02		0.03		0.02		0.03		0.02		NS		0.02		NS	
Z		0.02		0.03		0.02		0.03		0.02		0.04		0.02		0.04	
L		0.02		0.04		0.02		0.04		0.02		0.04		0.02		0.04	
SZ		0.02		NS		0.02		NS		0.02		0.05		0.02		0.05	
ZL		0.03		NS		0.03		0.06		0.03		0.06		0.03		0.06	
SL		0.03		0.06		0.03		0.06		0.03		NS		0.03		NS	
SZL		0.04		0.08		0.04		NS		0.04		NS		0.04		0.09	

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

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

ZSB	DAI	DTPA-Zn (mg kg ⁻¹)															
		15 DAI				30 DAI				60 DAI				90 DAI			

(+) ZSB	1.24	1.33	1.41	1.47
(-) ZSB	1.06	1.08	1.05	1.06

UNDER PEER REVIEW